THE METHOD OF ACTION OF RADIUM AND X-RAYS ON LIVING TISSUES

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BY

HECTOR A COLWELL

M.B., Ph.D., M.R.C.P., D.P.H.

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Awarded the Gorton Prize and Gold Medal of the British Empire Cancer Campaign 1921

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By the same Author

RADIUM, X RAYS, AND THE LIVING CELL
By HECTOR A. COLWELL, M.B., and Professor SIDNEY RUSS, D.Sc., F.Inst.P *(2nd Edition.)*

PRACTICAL BIOLOGICAL CHEMISTRY
Translated from the French of MM. BERTRAND and THOMAS.

ESSAY ON THE HISTORY OF ELECTROTHERAPY

INTRODUCTION TO THE STUDY OF X RAYS AND RADIUM
By HECTOR A. COLWELL, M.B., and C. P. G. WALMSLEY, F.R.C.S.

NOTES ON RADIUM THERAPY

X RAY AND RADIUM INJURIES
By HECTOR A. COLWELL, M.B., and Professor SIDNEY RUSS, C.B.E., D.Sc., F.Inst.P.

PRINTED IN GREAT BRITAIN

DEDICATED
TO
MY WIFE

PREFACE

It is now nearly twenty years ago since Professor Sidney Russ and I published the first collected account of the general action of Radium and X rays on living tissues The present work is likewise the first book of its kind and here I have endeavoured to give some account of the methods by which radiations produce their effects on cells tissues and neoplasms

The science of Experimental Radiology is still in its infancy notwithstanding the fact that a great deal of progress has been made in recent years but a vast amount of information has accumulated, and what is more it has given indications of the directions towards which future investigations may most profitably be directed The matter is not one of theoretical and academic interest but rather of intense practical importance especially as regards the radiation therapy of malignant disease The idea that the action of radiation upon cancerous growths is purely local is now discredited by many competent observers and clinical as well as experimental evidence points to a much more widespread effect

It is now over a year since this Essay was originally written, the manuscript was not available for publication until nearly the middle of August when not only were printing facilities almost suspended but I was otherwise employed on work which necessitated almost unremitting attention Nevertheless the greater part remains as it was written with the exception of the description of the work upon autolysis which has been considerably expanded In this as in many other pieces of work I have had the privilege of the collaboration of my friend Dr R J Gladstone F.R S E and it is to his kindness that I am indebted for permission to publish our joint work here before it has appeared elsewhere To all my fellow workers at the Barnato Joel Laboratories of the Middlesex Hospital I tender my sincere thanks for their kind help and especially to Miss D F Clophan and Miss Jean Boyd the former has helped me by contributing some of the illustrations and reading the proof sheets whilst

the latter rendered me inestimable service in transcribing the MS for submission to the Judges for the Garton Prize

To all who have allowed me to reproduce illustrations, I am deeply grateful and acknowledgements will be found in the list of illustrations

In conclusion I have to thank the Medical Research Council for a grant extending over several years during which most of my own work which appears here was carried out

My acknowledgements are also due to the British Empire Cancer Campaign, the holders of the copyright of this Essay for kindly permitting its publication

HECTOR A. COLWELL

BARNATO JOEL LABORATORIES
MIDDLESEX HOSPITAL
LONDON

March 1935

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INTRODUCTION

THE discovery of X rays by Röntgen in November 1895 followed by that of radioactivity by Becquerel in February 1896 may fairly be said to have opened a new era in science problems which had hitherto seemed of merely academic interest acquired practical importance and stimulated new fields of research. The fruits of this work are known to all, not only in the medical and surgical uses of X rays and radium and in the application of the former to industrial purposes but in the stupendous changes which have been wrought in the domain of chemistry and in the realization of the intimate relationship between physical and chemical phenomena As has been justly remarked by Bayliss it is impossible in the light of recent research to dissociate the two subjects of physics and chemistry It is a useful and indeed necessary practical convention to speak of the sciences as distinct but every advance in knowledge goes to establish their interdependence and we are already beginning to require explanations of chemical phenomena in terms of electrons

The chemical atom has been deposed from its former proud pre-eminence as being—in some eighty or more different forms —the ultimate structural unit of matter proton electron neutron and possibly other sub-atomic units have succeeded and in their turn are being subjected to the search lights of critical analysis Investigations upon colloids have opened up a vast mine of unsuspected wealth of results in the field of research, and experiments upon matter in the colloidal state have given consistent explanations of many fundamental reactions in biochemistry Since proteins are typical members of this group the importance of colloid chemistry in the present connexion is manifest

In what used to be termed the pure biological sciences progress has been no less marked the methods of tissue culture vital staining and micro-dissection have been productive of a marvellous harvest of results and have liberated us from what Michael Foster justly termed the pitfalls of carmine and Canada Balsam Depreciation of the older

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histological methods of examination by fixation and staining is not implied by this phrase these are neither obsolete nor obsolescent but essential for many lines of research Rather the old and the new methods are complementary one to another and a comparison of the results obtained by various methods has led to the elucidation of many doubtful problems and has allowed established fact to replace hypothetical speculation

The methods of micro-dissection have been widely extended and the foundations of a science of intracellular chemistry and physics have been well and truly laid

It is clear that the investigation of the actions of radiations upon living tissues is no light task that it covers an immense field and embraces a wide range of very different subjects It might seem almost impossible to correlate and coordinate such a diverse wealth of material but the attempt has been made and some at least of the resulting phenomena have received rational explanation upon a physico-chemical basis The problem is nevertheless, far from being solved and all that can be attempted here is to collect some of the results of experimental work and then to discuss the various ways in which they may have contributed in part at least to the solution of our problem.

Upon what seems a very common source of fallacy and one which in my opinion has tended to obscure even more a very difficult question I think it necessary to insist at the outset this is the idea that any one single explanation can be found for these highly complex and various changes such a view it seems to me is bound to be insufficient Too often has the attempt been made to explain the action of radiation in terms of the real or supposed action of the rays[1] upon some particular cell constituent—such as lecithin or cholesterol—or upon some particular organ or tissue—such as the blood vessels or the sympathetic nervous system Experimental findings must be the basis of our inquiry and when they appear discordant, the cause of the discrepancies must be sought out There are probably many elements of

[1] For purposes of brevity we shall often use the term β rays in speaking of the β radiations, without prejudice to older or later views upon their essential nature.

truth in the various hypotheses which have from time to time been put forward it is our task to coordinate these experimental results and, above all to remember that they are not necessarily mutually exclusive

There is yet a further point upon which caution appears to be necessary It is sometimes found that some particular explanation of a given series of events is in accordance with a simple mathematical statement and the conclusion is therefore reached that the explanation in question must be the true interpretation of the events under discussion Bayliss —than whom we need require no more competent authority on such matters—has already drawn attention to this possible source of error and has given the well known example of the rate of beat of the isolated mammalian heart with rise of temperature It has been found he says that this rate is directly proportional to the absolute temperature just as some simple physical phenomenon such as the expansion of a gas The conclusion might be drawn that no chemical process occurs in the heart and that the beat is a purely physical process which is absurd. Such explanations may be true they are not necessarily so

The most convenient and logical method of approach will probably be first to consider some points in connexion with the structure and physico-chemical behaviour of the normal cell then to deal with the action of radiations upon normal cells in the light of experimental data next to pass in review some of the metabolic changes resulting therefrom and, finally to assemble and re-examine our findings in the light of clinical experience

In view of their very limited range of therapeutic application it may be thought that in the following pages undue prominence has been given to the action of β radiations Their study nevertheless affords valuable information, especially as indicating the direction in which possible changes brought about by the X and γ-rays is to be sought In addition to this is of course the fact that both X and γ rays give rise to secondary electrons which are probably the essentially active agents in the production of many chemical and biological phenomena arising from exposure to these radiations

CHAPTER I

THE CELL

Even at the cost of repeating well known facts it may be desirable to give a brief account of the structure of a typical animal cell. With the vegetable cell we are not here concerned there are well marked differences both in structure and function between animal and vegetable cells The most prominent of these differences may be initially traceable to the fact that very early in the evolution of the vegetable as distinct from the animal phylum a wall of cellulose was secreted which is not represented in animal cells

For our present purpose a cell may be defined as an organized protoplasmic unit with a differentiated nucleus cytoplasmic inclusions and an external limiting membrane Summarized in tabular form these constituents may be expressed thus

(1) *Cell membrane*

(2) *Cytoplasm* and inclusions
- (*a*) Granules
  - microsomes
  - macrosomes
- (*b*) Mitochondria
- (*c*) Golgi apparatus
- (*d*) Chromidia

(3) *Nucleus* with nuclear membrane and nucleolus

The Cell Membrane

It was long a debated question whether a differentiated external cell membrane existed in typical animal cells The question has been answered in the affirmative by the methods of micro-dissection and the cell membrane shown to possess some remarkable properties which are probably of fundamental importance in the life of the cell.

In the first place may be mentioned the example quoted by Chambers of two pairs of mesenchyme cells observed in tissue cultures from the embryo chick. In the former pair the two cells were apparently completely separated except for a narrow connecting thread of protoplasm On puncturing

one of these cells with the micro-dissection needle a coagulation of the nucleus of the injured cell immediately occurred After a few seconds this was followed by coagulation of the nucleus of the uninjured and apparently separate cell The

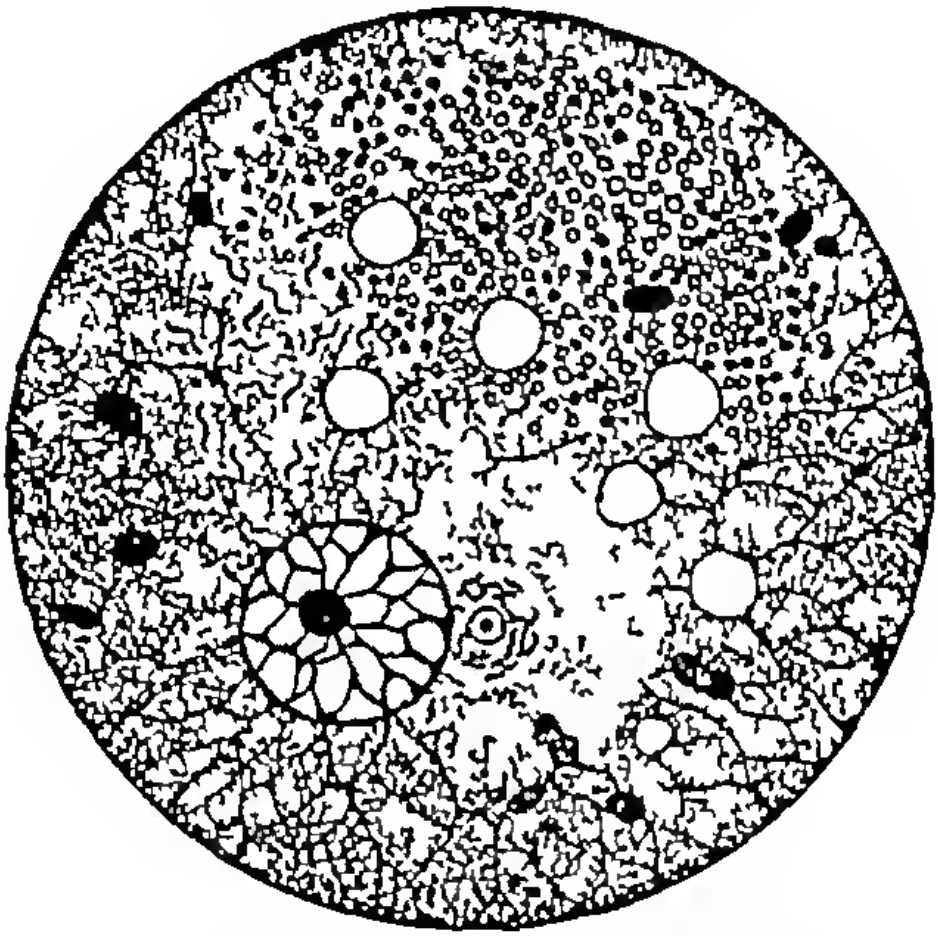

FIG 1 Diagrammatic representation of a cell highly magnified. The cell is limited by a cell membrane and contains a nucleus with a well defined nucleolus. The protoplasm of the cell body (cytoplasm) is composed of a finely granular matrix (hyaloplasm) traversed by a network of fibrils (cytoreticulum or spongioplasm) Near the nucleus is a clear body the centrosome or attraction sphere, containing a central body or centriole The centrosome is surrounded by a Golgi apparatus, which consists of fine thread like filaments imbedded in a sphere of clear hyaloplasm. From the centrosome granules radiate outwards into the surrounding protoplasm forming an aster The cell body contains also some free mitochondria, secretory granules, vacuoles and paraplasmic bodies.

The figure is a completely idealized one; various staining methods would be necessary to show the different structures indicated. (Gladstone)

effects of the injury had been propagated to the untouched cell in virtue of the protoplasmic continuity between them

In a second pair of mesenchyme cells the reverse type of conditions obtained Two cells were so closely contiguous

that they presented the appearance of a single binucleate cell. On injuring this mass of protoplasm as before an immediate coagulation of the nucleus adjacent to the site of injury occurred the cells responded by partially withdrawing from each other the presence of two investing cell membranes became apparent and only the one nucleus underwent coagulation

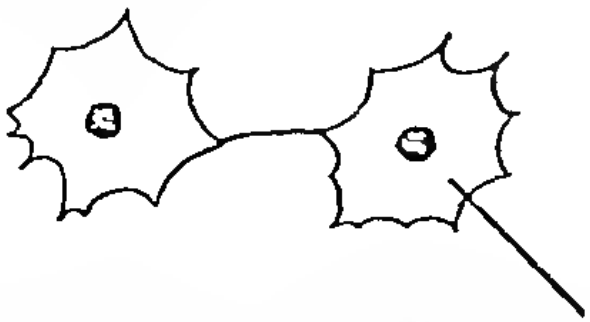

FIG 2. A pair of cells connected only by a narrow filament of protoplasm, yet on puncturing one of these cells by the micro-dissection needle the injury is transmitted to the other cell, as shown by the coagulation of the nucleus in both. (Cowdry's *General Cytology*)

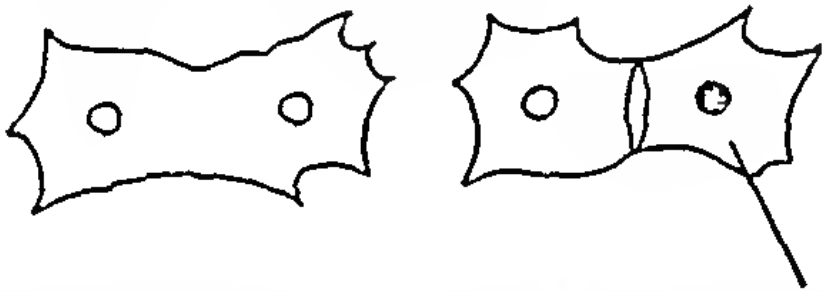

FIG 3. A pair of cells apparently forming a single binucleate protoplasmic unit; yet on puncturing one as before the cells separate and nuclear coagulation occurs only in the injured cell. (Cowdry's *General Cytology*)

This is a remarkably striking instance of the propagation of injury by protoplasmic continuity and of its restriction where cells are separated by distinct cell membranes each cell acts as a separate unit and this independence is a function of the integrity of the cell membrane Incidentally the fact is emphasized that optical differentiation of the cell membrane may be absent or obscure

If the cell membrane of an echinoderm ovum is torn one of two things may happen according as the tear is slight and gradual or sudden and extensive If the puncture be very gently done a portion of the cell with its surface membrane

can be drawn out and finally separated. The extended portions are withdrawn and the cell membrane closes the gap in the ovum. (Lewis 1922)

A similar occurrence can be noted in the amœboid movements of some protozoa. If a portion of the protoplasm (and cell membrane) adheres to the medium upon which it is moving the adherent part is left behind attached at first to the rest of the cell by a strand which becomes finer and finer as the separation increases until eventually rupture is complete. The broken strand attached to the main cell is slowly withdrawn, the protoplasmic fragment retracted

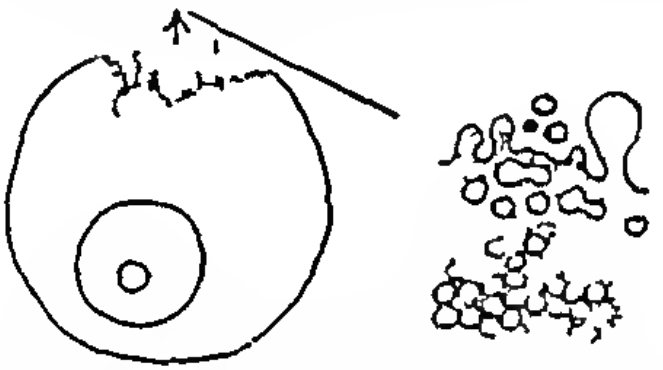

FIG. 4. To show the disintegrating effect produced on the cytoplasm of a cell (star fish egg) by rough tearing of the cell membrane. On the right, an enlarged view of the disintegrating cytoplasm. (Cowdry's *General Cytology*)

into the body of the cell and the gap closed by the cell membrane.

If on the other hand the cell membrane be roughly and extensively torn disintegration of the cytoplasm occurs immediately at the site of injury and rapidly spreads. Attempts at closure of the breach by the formation of local surface films have been observed but they are generally abortive.

A further characteristic of the cell membrane is its semi permeable nature as demonstrated by the micro-injection methods of Chambers (1922). From these experiments it can be seen that electrolytes and dye-stuffs dissolved in the surrounding medium do not tend to enter the uninjured cell. When introduced beneath the surface film by the micro-injection pipette they exert their characteristic effects. These effects differ according to the chemical nature of the substances introduced. Thus if basic dyes—e.g. Janus green, methylene blue, neutral red—are injected, they produce a

local stained coagulum which disintegrates and is dissipated throughout the cytoplasm If on the other hand the dye is acid in character—e.g trypan blue trypan red eosin, acid fuchsin, alizarin—no coagulum occurs but liquefaction If the amount injected is sufficient death occurs but when used in small enough quantity to allow of recovery the dye spreads through the cell and diffuses out

The point to be observed is that the *uninjured* cell membrane is of the semi permeable variety A very striking example of this is to be found in the chemical analysis of the sodium potassium content of blood plasma and red corpuscles made by Abderhalden In the plasma the sodium content is high while the potassium content is *nil* in the corpuscles the potassium content is high and the sodium content minute

	Red Corpuscles Parts per 1,000	*Blood plasma Parts per 1 000*
Na		4 358
K	3 326	0·254

Another well known example is that sodium and chlorine ions are practically absent from muscle fibres but present in the blood plasma

A brief consideration must now be given to the probable structure of the cell membrane It is regarded as formed by the surface condensation of certain elements of the protoplasm and a characteristic property is that it is found to be permeable to objects of visible dimensions as seen, for example in the ingestion of bacteria in the process of phagocytosis or in the ingestion of other organisms by the amœba In these cases permeability is caused by direct local rupture of the film by the particle in question, after which its integrity is restored. In the case of ions or of colloidal micellæ penetration can only take place if these are of sufficiently small dimensions to pass through the pores in the film The condition in the living cell is however found to be of a more complex character since the permeability of the film to ions or colloidal micellæ is observed to differ when the cell is in the active or the resting state

The chemical nature of the cell membrane is in all probability one of great complexity but experimental evidence points to it being a system involving at least two essential phases One is a watery solution of protein, the other is a lipoid phase The permeability of the cell membrane is thus a function of the activity of the cell and the conditions obtaining in different circumstances may be briefly summarized in tabular form as follows

In all states of the cell	*In the resting state of the cell.*	*In the active state of the cell.*
The membrane is PERMEABLE to substances soluble in water *and also* in oils or lipoids. Such are: urea, oxygen, carbon dioxide, some ammonium salts, alcohol, and chloroform.	The membrane is IMPERMEABLE to most salts (except certain salts of ammonium), to glucose and to amino-acids.	The membrane is PERMEABLE to salts, glucose, and amino acids.

Bayliss, from whom the above facts are taken, proceeds to say

The facts mentioned support the suggestion made by Clowes that the membrane is a system of two phases, a watery solution of protein and a lipoid phase In the resting state, the lipoid phase is the external or continuous one so that the pores between the elements of the watery phase are filled with lipoid and can only be passed by substances soluble therein In the state of activity the position of the two phases is reversed Here the watery phase is now continuous, so that any substance soluble in water even if insoluble in lipoid, can now pass through

It has been experimentally shown by Clowes that artificial systems of this character can undergo such a reversal of phase

Accepting this view of the constitution of the cell membrane there are two conditions necessary for a substance[1] to be able to penetrate it If of molecular or ionic dimensions it must be soluble in what happens at the time to be the continuous phase of the film but to pass between the elements of the disperse phase if the substance is of the order of size of a colloidal particle it must be sufficiently small to pass

[1] That is, of course, apart from the mechanical temporary rupture referred to previously in the case of microscopically visible objects, such as bacteria.

through the pores In considering molecular or ionic dimensions the associated water molecules must also be taken into account

It will be noted that glucose is an important source of energy for the active cell while amino acids are the building stones out of which the cell protoplasm is to be elaborated The membrane accordingly becomes permeable when these substances are particularly needed Similarly potassium is necessary for the carrying out of the specific functions of the

FIG 5. To show the effect of membrane permeability on distribution of ions In the left hand figure the membrane is impermeable to both kations and anions. In the right hand it is permeable to anions, which pass through, but not to kations. Owing to electric force of attraction, the anions form a layer on the surface of the membrane

muscle-cell, and it is precisely in this state of activity that the membrane is permeable to the potassium ion.

There is a further condition which arises from the permeability or impermeability as the case may be of the cell membrane to the ions of dissociated electrolytes In this case it resolves itself into a question of the distribution of electric charges

If as suggested in the diagram it is the kation which is unable to escape it will be retained inside the cell while the anion passes through the membrane Owing to the force of electrostatic attraction, the anions will tend to form a layer on the outside of the cell. Anything interfering with the permeability of the membrane will obviously tend to interfere with these conditions In normal muscular activity for instance it has been established that there is actual increase in the permeability of the membrane the kations hitherto unable to pass through will now be able to do so and the result will be the passage of an electric current

It is probably evident from the preceding account that the cell membrane is capable of playing a very important part in the metabolism of the cell, and consequently that any interference with its normal properties will lead to alterations

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in the cell metabolism As Jacobs observes there is a universal property of cells to limit in a means often very complex the diffusion of dissolved substances

We shall later on show that radiations are capable of affecting the cell membrane and in one case at any rate of producing such changes as to allow of the passage outwards of a substance to which the membrane is normally impermeable

The Cytoplasm and Cytoplasmic Inclusions

The cytoplasm itself is a colourless and translucent substance often containing imbedded granules and vacuoles examined under dark-ground illumination it is therefore optically heterogeneous The granules vary much in size and character according to the type of cell examined. Of these there is one type the *microsome* rather less than 1 μ in diameter, which appears to be universally present notwithstanding its minute size it is plainly visible owing to its highly refractile character Microsomes are of special histological importance since under dark-ground illumination they give the appearance of luminous discs which may or may not show Brownian movement In low states of viscosity this is marked while with sufficiently high degrees it disappears

The *macrosomes* measuring from 3 to 4 μ in diameter approach so closely in refractivity to the surrounding cytoplasm that they are much less evident than the microsomes It is quite probable that they are nutritive in character They are probably fluid and tend to run together when cytolysis occurs Other cytoplasmic inclusions are well marked such as oil globules secretion granules deposits of glycogen and other materials concerned either in secretion or nutrition these vary in nature size and number according to the type of cell under examination.

The *mitochondria* Owing to the easily soluble character of these bodies in most ordinary fixing reagents their study is one of the comparatively recent developments of modern histology The amount of literature which has been accu

mulated on the subject is however enormous and the diverse functions which have been attributed to mitochondria is evidenced by the number of names which from time to time have been given to them Cowdry (1918) recorded about ninety of these latter while the number of substances with the metabolism of which they have from time to time been associated is upwards of eighty The association of many or rather most of these is entirely fantastic and only one or two are worthy of serious consideration

Varying in form from relatively long filaments to almost granular shape they however appear to be present in every type of cell hitherto examined whether animal or vegetable with the possible exceptions of non nucleated red blood corpuscles and of bacteria. It is also noteworthy that in many spermatozoa the mitochondria and nuclear elements of the original spermatozoon are the only parts which enter the ovum at fertilization. Their practical ubiquity in cells of such varied types would point to their importance in cell metabolism Two micro-chemical reactions particularly distinguish them Firstly their vital staining by the dye Janus green B by this they are first of all stained bluish green, but subsequently this undergoes reduction and they become pink. The reaction is not only characteristic but highly specialized since very slight chemical differences in the dye used will cause failure of the reaction The second point is their ready solubility in such reagents as acetic acid or alcohol for their fixation special reagents especially those of the bichromate-formaldehyde type must be used

It has already been said that they vary much in form but although the length varies enormously in different cells and in the same cell under different conditions of activity their diameter appears to be remarkably constant in the same type of cell This would rather point to a certain limitation of depth wherein they are best able to manifest their activities It is obvious that structures of this kind must expose relatively large surfaces and since extent of surface facilitates chemical reactions this indicates that their function is connected with the chemical phenomena of the cell It is said that in very young embryos of the same type most of the

cells contain approximately the same number of mitochondria subsequent specialization of the cells leads to alterations in form and distribution, though in the same cell types considerable uniformity is maintained To Regaud is due the interesting observation that with the evolution of spermatogonia to more matured form the mitochondria show a marked increase in resistance to the action of dilute acetic acid.

For their chemical structure the evidence is admittedly largely negative they do not give Macallum's reaction for iron nor do they stain with Sudan III From their behaviour towards ordinary fixing reagents and from their staining powers when so fixed the general conclusion has been reached that phospholipins probably enter largely into their composition. The evidence in favour of this view of the chemical constitution of mitochondria may be summarized under the following six heads (Cowdry 1918)

1 They are soluble in alcohol ether chloroform and dilute acetic acid. They do not stain with Sudan III or Scharlach R and are only sometimes blackened by osmic acid.

2 With Millon's reagent they do not give the characteristic reaction which is given by proteins in which there is a hydroxylated benzene nucleus This absence of reaction is in marked contrast to the strongly positive reaction given with Millon's reagent by zymogen granules. It is however admitted that the Millon reaction *might* be positive and yet not strong enough to show up against the stained cytoplasm in these extremely fine rod like or filamentous structures Cowdry's experiments failed to give a definite xanthoproteic reaction. No reactions characteristic of polysaccharide groups have been obtained

3 Artificial mitochondria have been made in different salt and albumin solutions and have been found to give similar staining reactions

4 They appear to melt or dissolve somewhere about 48 –50 C

5 There appears to be a correspondence between the

number of mitochondria seen and the phospholipin content as determined by chemical analysis

6 Injections of lecithin given to fowls or pigeons have been said to increase the number of mitochondria present in the oöcytes

It has been suggested that they largely function in the process of respiration certainly with the development of haemoglobin in the series of cells which lead up to the formation of the mammalian red blood-corpuscle they gradually diminish as the haemoglobin content increases A similar diminution is noticed in vegetable cells with the gradual increase in the formation of chlorophyll A special connexion with oxidation was first suggested by Kingsbury no evidence has been forthcoming to refute it and all observers who have specially investigated the point think that their results endorse this view Thus Mayer Rathery and Schaeffer (1914) admitting the phospholipin character of mitochondria consider that owing to their power of auto-oxidation this chemical foundation would be suitable for the occurrence of processes of oxidation and reduction. They further consider that in the mitochondria are unsaturated fatty acid and ethylidene groups which have considerable affinity for oxygen It is further pointed out that such reagents as alcohol chloroform and ether while dissolving mitochondria also depress respiratory oxidations while their universal presence in the various types of cell is also held to point in the same direction

From experiments with tissue cultures the same conclusion was reached by Lewis (1915) and Cowdry (1916) regarded his experiments with Janus green as supporting the same view

Regaud has put forward his electosome theory namely that mitochondria select and act upon certain substances in the cytoplasm according to the specific function of the cell under discussion In some cells they seem very much more highly sensitive and more easily destroyed than they are in others In the cells of the central nervous system they show extraordinary stability Certainly the great surface development of structures containing such chemically active bodies

cells contain approximately the same number of mitochondria subsequent specialization of the cells leads to alterations in form and distribution, though in the same cell types considerable uniformity is maintained To Regaud is due the interesting observation that with the evolution of spermatogonia to more matured form the mitochondria show a marked increase in resistance to the action of dilute acetic acid.

For their chemical structure the evidence is admittedly largely negative they do not give Macallum's reaction for iron nor do they stain with Sudan III From their behaviour towards ordinary fixing reagents and from their staining powers when so fixed the general conclusion has been reached that phospholipins probably enter largely into their composition. The evidence in favour of this view of the chemical constitution of mitochondria may be summarized under the following six heads (Cowdry 1918)

1 They are soluble in alcohol ether chloroform and dilute acetic acid They do not stain with Sudan III or Scharlach R and are only sometimes blackened by osmic acid

2 With Millon's reagent they do not give the characteristic reaction which is given by proteins in which there is a hydroxylated benzene nucleus This absence of reaction is in marked contrast to the strongly positive reaction given with Millon's reagent by zymogen granules. It is, however admitted that the Millon reaction *might* be positive and yet not strong enough to show up against the stained cytoplasm in these extremely fine rod like or filamentous structures Cowdry's experiments failed to give a definite xanthoprotein reaction No reactions characteristic of polysaccharide groups have been obtained

3 Artificial mitochondria have been made in different salt and albumin solutions and have been found to give similar staining reactions

4 They appear to melt or dissolve somewhere about 48 –50 C

5 There appears to be a correspondence between the

number of mitochondria seen and the phospholipin content as determined by chemical analysis

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as the phospholipins is in favour of surface action, which is possibly specialized to attain different ends in different types of cell.

When subjected to various toxic agents they undergo change of form thus in phosphorus poisoning Scott has shown that they lose their filamentous character form

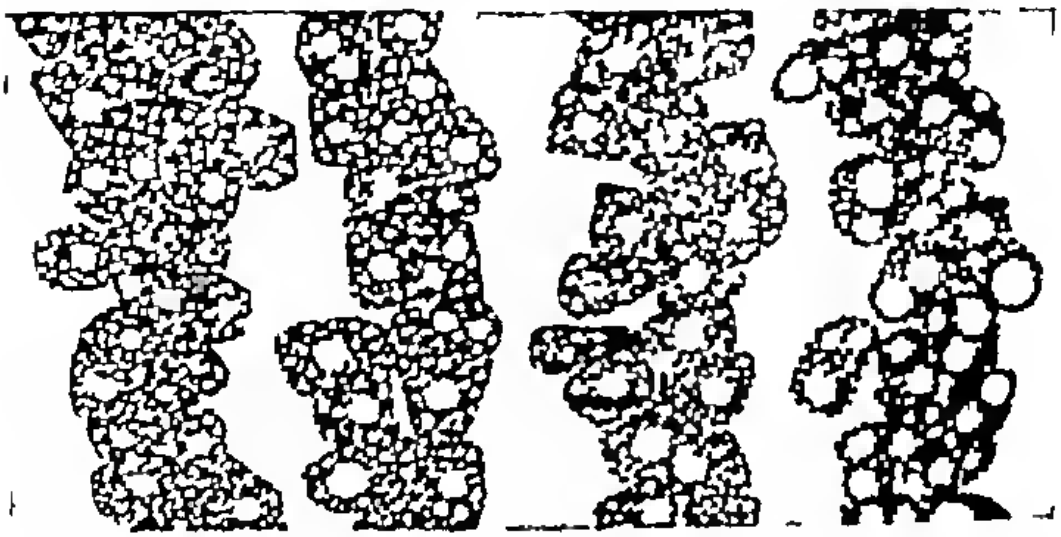

Fig 6 Variations in form of mitochondria due to cell activity (Kater).

Sections from the livers of cats under different conditions of hepatic activity On the extreme left is shown the appearance of the mitochondria when the animal has been fasting for 24 hours; the next section is from a similar cat which had also been injected with insulin; the third section is from a fasting cat which had been anaesthetized with ether for two hours while the fourth is from a fasting cat injected with adrenalin. All specimens prepared by Regaud's method. Magnification × 844 ($\frac{1}{12}$ inch oil immersion).

Insulin, adrenalin, and ether anaesthesia are all agents which disturb the glycogen-glucose equilibrium. (Kater *Anat. Record*, 1931)

clumps break up into granules and finally give rise to droplets suggesting fatty degeneration. This tendency to break up into minute spherules under adverse conditions suggests an attempt at further increase of surface

Alterations in form occur with the stage of activity of certain cells for instance gland cells but in this connexion Fry (1930) has drawn attention to the necessity for uniform and standardized histological technique Alterations due to changes in cell activity have been reported by a great number of workers including Cramer and Ludford (1926) Morley and Smith (1930) and Kater (1931) the last-named draws attention to the extensive literature which has developed around

the subject It is worthy of note that Kater arrives at the conclusion that alterations in the shape of mitochondria depend rather on the degree of metabolic activity than upon the special functions of particular cells This may point to a specific function in connexion with secretion it seems however an equally tenable hypothesis that variations in metabolic activity may be accompanied by changes if they are specifically concerned in oxidation and reduction processes

The question is not yet solved but it can be said with a fair degree of certainty that the mitochondria are the site of important chemical reactions As will be seen later they are remarkably sensitive to radiations some authorities consider them by far the most sensitive of all the differentiated cell contents

The Golgi apparatus The Golgi apparatus named after the Italian neurologist to whom its earliest mention is due (1898) has like the mitochondria been the subject of intensive study in recent years and like them also requires special histological methods for its demonstration In properly prepared specimens it appears as a kind of more or less complicated knot situated in the cytoplasm It is certainly present in a great number of metazoan cells Gatenby stated that it was always present but Cowdry expresses some reserve upon the point It appears however to be present in all the cells of developing embryos while one remarkable characteristic is its situation in certain cells In the cells of such glands as the salivary glands and the pancreas it is situated between the nucleus and the free edge bordering upon the lumen. In the thyroid on the contrary it varies in position being sometimes next the lumen containing the colloid secretion and sometimes on the side remote from this The alteration in situation has been held to indicate differences in the passage of the products of thyroid metabolism—i e towards the colloid filled lumen on the one hand or towards the blood vessels and lymphatics at the periphery of the alveolus on the other Cajal observed that in developing embryos the Golgi apparatus is always present in the cytoplasm between the nucleus and the periphery The chemical nature of its constituents is not yet established

a lipoid character has been suggested and its physical character whether solid liquid or gel or a combination of two or of all three is still a question which must be regarded as unsolved. So far as functions are concerned we are still in the dark There are the suggestive changes of position in the cells of the thyroid gland and the constant position between the nucleus and the cell border next the lumen in such structures as the salivary glands equally however a Golgi apparatus appears a very constant feature in cells of all types Pathological conditions have hitherto not shed much light upon the matter

Chromidia and chromidial substance are terms which are somewhat loosely used and cover such cell inclusions as the Nissl substance of nerve-cells and the basophilic material of glands It has been understood to mean extranuclear fragments of chromatin here the difficulty of the precise limitation of the term chromatin arises Practically under the term chromidia are included a variety of granules distributed through the cytoplasm and showing a marked affinity for basic stains Probably a considerable variety of dissimilar structures are included under this common heading The Nissl substance of nerve-cells is relatively insoluble in alcohol, gives micro-chemical tests for iron and is not seen in the living cell.

The Nucleus

The cell nucleus is seen in two well marked and distinct states—when it is dividing and when it is resting The latter term has the sole virtue of brevity to recommend it and it is better to refer to the nucleus when not undergoing division as interkinetic which makes no *a priori* and almost certainly incorrect assumption as to what its functions at the time may happen to be Probably resting is about as inaccurate and misleading a term as could well be chosen.

The interkinetic nucleus shows a well-defined nuclear membrane separating the nucleoplasm from the cytoplasm Within the nuclear membrane are one or more nucleoli, and with these exceptions the nucleoplasm appears structureless in unstained specimens and homogeneous when examined by

dark-ground illumination. Normally it is of fluid consistency though readily setting to a firm gel Should the nuclear membrane be ruptured as by the micro-dissection needle the cytoplasm immediately surrounding the nucleus rapidly disintegrates and liquefies within the nucleus itself the immediate result of the injury is the dissolution of the nucleolus Chambers has succeeded by means of the micro-pipette in aspirating the fluid contents of the nucleus of one cell and injecting it into the cytoplasm of another In this case also the cytoplasm undergoes liquefaction and disintegration A remarkably interesting fact is that the nuclear substance if allowed to remain more than five or ten *seconds* in the micro-pipette becomes innocuous so that as Chambers remarks the operation must be performed rapidly

The familiar nuclear network seen in fixed and stained specimens is not seen in the living interkinetic nucleus but is readily made to appear by the action of reagents or sometimes merely by rough treatment

The dividing nucleus shows well marked structural features both in the living state and in fixed and stained preparations These as is well known vary in appearance according to the particular phase of mitosis under examination

Very interesting in this connexion is the experimental work of Chambers upon the growing spermatocyte of a species of grasshopper (*Dissosteira carolina*) The nucleus of this type of cell is apparently optically homogeneous with the exception of the nucleoli. Upon injury instead of the formation of an anastomosing network, the strands which appear develop into more or less granular filaments and give the appearances characteristic of a typical prophase nucleus Loops of this filamentous structure can be drawn out by the micro-dissection needle and stretched, so that its finer structure becomes manifest when it is shown to consist of a hyaline core with granules embedded in the periphery When the loop is stretched the fibres become narrowed while the granules are more widely separated.

The nuclear injury it is probable is not responsible for the formation *de novo* of either the network of the interkinetic nucleus or of the prophase-type of chromatin filament found

in the spermatocyte Rather is it probable that they were actually present at the time of the injury though invisible owing to optical homogeneity with the rest of the nucleoplasm Injury to nuclei in which visible stages of mitosis are already present leads to an acceleration of the process usually with the formation of a typical mitotic figure These facts suggest the probability that the nuclear network seen in fixed specimens of the interkinetic nucleus is not entirely an artefact but has its structural though generally invisible counterpart in the unstained specimen

The nuclear phases occurring during mitosis—prophase, metaphase anaphase and telophase—are too well known to need description During the metaphase the nucleus becomes more or less fusiform instead of rounded the hitherto distinct nuclear membrane disappears while the chromosomes collect at the equator of the spindle That the spindle is a true anatomical structure has been demonstrated by micro-dissection and it has the power of setting to an irreversible gel upon mechanical injury

CHAPTER II

CHEMICAL ACTIONS OF THE RADIATIONS

VERY soon after the discovery of X rays and of radioactivity experiments were started to investigate their possible chemical effects In the case of X rays these were largely directed to attempts at finding some simple method of dosage In the case of radium the investigation took rather different lines and doubtless owing to the large output of α radiation, its effects especially in gaseous systems attracted a large amount of attention. It must be admitted that much of this early work was of very unequal value to a large extent this was owing to the difficulties inherent in the study of all new subjects and especially to the lack of precise measurements and—more especially in the case of radium—to a want of knowledge of the effects of screenage As is well known the investigations on the effects of α-particles were crowned by Rutherford's discovery of atomic disruption

But it is not with the action of α particles that we are concerned in the present connexion except that the experimental work carried out with them showed that their ordinary chemical effects were mainly determined by the changes in ionization which they produced

The ordinary chemical reactions of X rays and of the β and γ-rays from radioactive substances are considered by Lind to be due to the same initial cause—ionization. At all events this view which is strongly corroborated by experiment offers a coherent explanation of the colloidal and intramolecular changes which are set up by the radiations

Space can only be found here for consideration of some of the outstanding results of the chemical actions of radiations which have a direct bearing upon their biological effects As occupying a more or less intermediate position between typically physical and typically chemical phenomena we shall first consider some of the effects of radiations upon colloids since cell protoplasm is very largely a carefully adjusted system of colloids the importance of the subject for our present purpose is manifest

Colloids

Among the earliest experiments upon the action of radiations on colloids were those of Hardy (1903) and of Henri and Meyer (1904) Experimenting with two samples of serum globulin rendered acid and alkaline respectively by means of dilute acetic acid and ammonia Hardy showed that exposure to α radiation caused the alkaline specimen to set to a firm gel in three minutes while the acid became clearer The explanation is that the positive charges carried by the α particles neutralized the negative charges of the disperse phase in the alkaline mixture thus leading to gel formation In the acid mixture the positive charges carried by the elements of the disperse phase have their action reinforced.

Henri and Meyer using inorganic hydrosols found that when these were exposed to β radiations the positive colloids were rendered much more readily precipitable by traces of electrolytes Here the negative charges carried by the β-particles neutralized the positive charges in the disperse phase and so facilitated precipitation in the presence of minute amounts of electrolytes

Colwell and Russ (1912) investigated the action of X rays upon organic colloids such as egg white serum and starch. In all of these a marked diminution of viscosity occurred and in the case of starch a partial conversion into dextrin was also noted In one experiment where irradiation had been allowed to proceed for 8½ hours at least 5 per cent of the starch had undergone this conversion

Fernau and Pauli (1915–22) carried out a number of researches upon the action of radium radiations using 220 mgm. of $RaCO_3$ sealed in a glass tube 1 1 mm in thickness by which 80 per cent of the β and 1 per cent of the γ-radiations were absorbed Native proteins and colloidal cerium hydroxide were among the substances investigated. The effects of varied hydrogen ion concentration were also made the subject of inquiry Varying differences in the state of aggregation of the sols were observed in the course of these experiments.

Recent work of Crowther and Fairbrother Between 1927 and 1930 the action of radiations upon colloids was reinvestigated

by Crowther and Fairbrother In a preliminary series of experiments with simple hydrosols of elements they found that these were relatively stable to X rays and required very large doses to produce appreciable change Positively charged colloids were found to be coagulated, while negatively charged colloids had their stability increased. The authors considered it probable that the coagulation was brought about by the ionization produced in the diffuse double layer surrounding the particles

A second and quantitative investigation was next made of the viscosity changes produced in cerium hydroxide sols by the action of X rays In sols of *low* concentration, and with increasing X ray doses the viscosity decreased to a minimum then rapidly increased and the sol set to a rigid gel. In more concentrated sols the spontaneous increase in viscosity which occurred when the particles were discharged masked the mutual decrease With increasing age the sols became more sensitive to X rays and the doses necessary to produce the maximum decrease in viscosity and to cause gel formation were much smaller thus indicating that the charge upon the particles was decreasing

When the state of minimum viscosity was reached, the sol set spontaneously to a rigid gel within the course of a few hours An X ray dose sufficiently in excess of that required to produce the maximum decrease caused the sol to set to a gel immediately

In a third communication the same authors extended their quantitative observations upon the action of radiations. With the details of their quantitative experiments we are not here concerned but they further discussed the question as to whether the action upon the sol was a direct ionization effect or due to secondary changes set up in the continuous phase It had indeed been suggested by Fernau and Pauli that these phenomena might be due to the formation of minute traces of hydrogen peroxide Thus is admittedly produced when water is subjected to β radiation but even with large doses of X rays no evidence of hydrogen peroxide formation could be detected even when the most delicate tests were applied

To throw light upon this subject Bredig sols of copper

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were made by sparking between copper electrodes under ethyl alcohol and also under amyl alcohol From both of these sols the copper was precipitated by the action of X rays. Traces of water would certainly be present in both of these alcohols but it was thought that when only such minute traces were present the exposure necessary to produce the requisite amounts of hydrogen peroxide would at any rate have been greatly prolonged This was not found to be the case.

A further series of tests were then carried out upon sols of silver resinate in oil of lavender and in benzene Here water if present could only have been so in the minutest traces Both sols however were precipitated by exposure to X rays The general trend of the evidence is therefore distinctly *in favour of a direct action* whereby the *colloidal* particles have their charges neutralized by the ion formed in the double layer around them. Crowther's general conclusions may be best given in his own words

> There remains the fact, for it seems to be a fact that only sols in which the particles are positively charged are coagulated either by X or beta radiation. Hardy for example found that positively charged globulin was coagulated by beta radiation but negatively charged solutions of the same substance were unaffected. Wels and Thiele report the same result, using X radiation In the course of the last two years we have examined a large number of sols, with the result that in no case have we been able to coagulate a negatively charged colloid, and in no case have we failed to produce coagulation where the particles of the sol were positively charged. With sols, such as silver iodide which can be prepared with the particles either positively or negatively charged the former are coagulated, the latter are apparently unaffected by the radiation
>
> It seems to me that the one-sidedness of the effect may be connected with the different nature of the initial positive and negative ions The positive ion is never smaller than an atom. The negative ion begins as a free electron, and in a gas persists in this state for an appreciable fraction of its existence Our knowledge of the behaviour of ions and electrons in a liquid medium is very scanty but it is not improbable that a similar state of affairs exists there also
>
> We now suppose that the colloid particles are surrounded by a closely adherent film of molecules of the solvent There is considerable evidence in favour of the view that the surface along which slip takes place when the particle moves under a field is entirely within the solvent and not between the solvent and the particle and Fairbrother's observation that the size of the colloidal particles decreases

as they lose their charge is additional evidence in favour of the supposition. If the colloid is electro negative it attracts the positive ions formed by the radiation in the double layer surrounding it The positive ion, however being molecular will be unable to penetrate the protecting skin[1] of solvent molecules which surrounds the colloid particle, and remains outside where it is either neutralized or takes the place of one of the electrolytic ions which form the outer shell of the double layer

If, however the particle is electro-positive so that it attracts the negative ion, the latter being in an electronic state and being in an electric field of the order of 105 volts per cm., will be able to penetrate the protective skin of solvent molecules and so to neutralize the charge on the particle itself An alternative suggestion, which experimentally is probably indistinguishable from the first is that the electron, by entering an atom on the outer face of the skin, causes the liberation of an electron from an atom on the inner surface in contact with the particle We have not at present sufficient knowledge to enable us to apply numerical tests to this hypothesis. It seems, however to be the one which most adequately explains our experimental results on the action of ionizing radiations on colloidal solutions.

Fairbrother (1928) investigated the viscosity changes in egg white under the action of radiation as part of the general scheme of research upon colloids carried out by Crowther and himself

The radiation was derived from a Shearer tube with molybdenum anticathode excited by an induction coil fitted with a mercury break. The voltage throughout the experiments was 55 000 and before reaching the fluid under examination the rays passed through an aluminium window 0·1 mm and a glass slip 0·23 mm in thickness Although the radiation was heterogeneous the beam reaching the albumin sol consisted largely of characteristic molybdenum K radiation ($\lambda = 0\,717 \times 10^{-8}$ cm)

The liquid was exposed in a small quartz vessel with a surface measurement of 28 mm ×0 mm and a depth of 0 mm This was covered by the glass slip previously mentioned 0 23 mm thick which was hermetically sealed over the dish with paraffin wax. In all the experiments the quantity exposed was 1 c c which filled the dish to a depth

[1] The term skin as applied here is, I think, open to objection. The quotation is given without alteration. H A. C.

of 4 mm As a control, a sample was placed in an exactly similar dish and sealed down in the same way the viscosity determinations of the experimental sol were always compared with this control sample and not with the stock solution This procedure eliminated sources of atmospheric contamination on the one hand and any possible changes due to the sealing process on the other

The viscosimeter consisted of a capillary tube with an intermediate bulb of 1 0 0 capacity and a reservoir at the base The fluid was sucked up from the reservoir and the time taken for it to fall from a mark above the bulb to one below it gave an arbitrary value of the viscosity The difference between the time taken by the exposed and control samples divided by the control time gave the fractional change in viscosity this figure was plotted against the corresponding doses of X rays

For the preparation of the egg white sol, the white of a new laid egg was taken, shaken into a foam and allowed to stand overnight In the morning the clear liquid had settled while the extraneous material remained suspended in the remainder of the foam

The intensity of radiation was measured by means of an air-gap ionization chamber The rays passed through an opening 10 mm. × 5 mm. cut in a lead screen and ionized a volume of air between two plates each 2 cm square The volume of ionized air was 1 0 0 To the insulated plate were connected a microfarad condenser and a gold leaf electroscope The radiation necessary to charge the microfarad condenser to a P.D of one volt was taken as the unit of dosage and the time necessary to administer this dose was about twelve minutes

Comparing this with a therapeutic measure of dosage it was found that one such unit was equivalent to 3 000 e, where e is Friedrich's unit The distances of the ionization chamber window and the surface of the liquid from the focal spot were 11 23 cm and 3 70 cm respectively Hence the unit dose given to the liquid was $(11\ 23)^2/(3\ 70)^2 = 9\ 4$ times that measured in the ionization chamber This is equivalent to a therapeutic dose of 28 200 e, or about 23 times that necessary for the production of erythema on the skin

The experimental results are shown in the accompanying graphs where the ordinates represent percentage decrease in viscosity and the abscissae the doses of X rays measured in the above-mentioned arbitrary unit. From Curve I it is seen that the viscosity decreases rapidly the rate of decrease at first becoming gradually less until a stationary condition is reached after which it decreases to a constant minimum

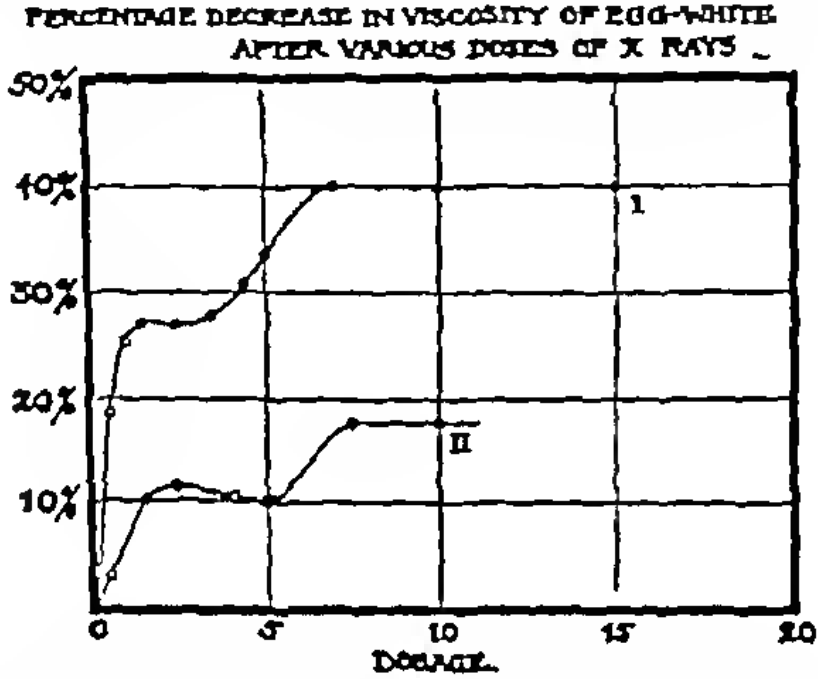

Fig 7 Graphs showing percentage decrease in viscosity of egg white after various doses of X rays. (Fairbrother *Brit J Radiol.*, 1928)

In this case the maximum decrease is seen to be 40 per cent (Curve I)

A different stock of egg white prepared in exactly the same way gives the results plotted in Curve II. The percentage decreases here are not so great as in Curve I but it will be seen that the maximum decrease in both cases is produced by the same dose—about 7 5 of the experimental units before mentioned. Also in this case the rate of decrease in viscosity not only becomes zero but changes sign so that there is an increase in viscosity between 2 5 and 5 of the experimental units

In a third experiment (Curve III) the effect of the addition of sodium chloride was studied To 10 c c of the stock albumin used for the experiment from which Curve I is

drawn, 0 1 gm of pure NaCl crystals were added. They dissolve readily in the protein sol and cause no change in its viscosity The kink seen in Curve I is absent, and the maximum viscosity decrease now reaches only 32 per cent.

Effect of irradiation on coagulation An irradiated sample in which the maximum (40 per cent) decrease in viscosity had

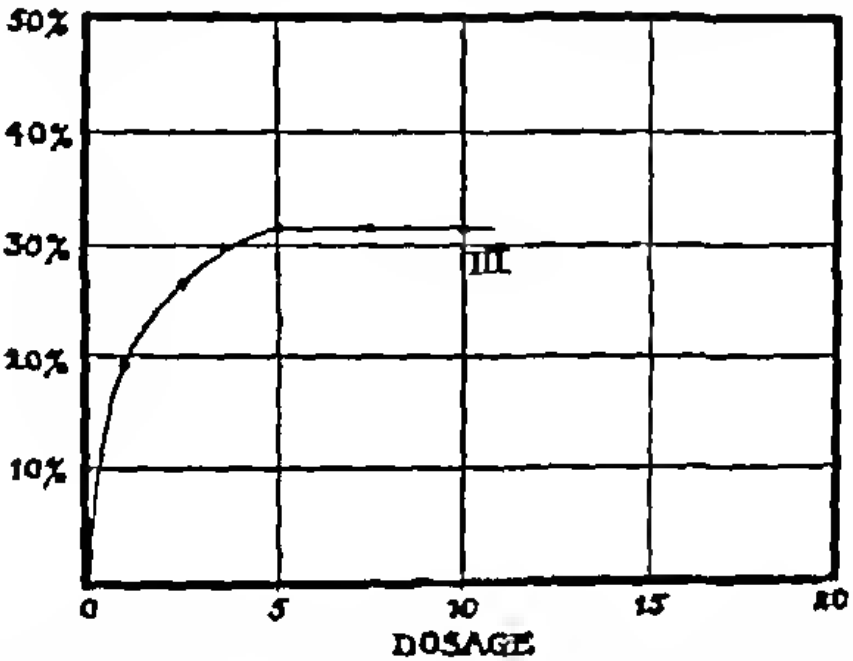

FIG 8. Graph showing effect of addition of NaCl upon viscosity changes produced by irradiation of egg white. Compare Curve I, Fig 7 (Fairbrother *Brit J Radiol.* 1928.)

been produced was placed in a test tube and this, together with a control was warmed up to 01 C in a water bath. When this temperature was reached the control became turbid and rapidly set to an opaque gel. The irradiated sample on the other hand while turning milky did not set until fully five minutes after the control, thus showing that when irradiated egg white is maintained at the control tem perature setting is quite markedly delayed

The author points out that egg white used in these experi ments was a mixture of albumin and globulin and that therefore Curves I and II might be summation effects produced by the radiation upon these different constituents and he considers that the addition of sodium chloride might have caused a partial salting-out of the globulin which would account for the simpler form of curve obtained in this case

No mention of the occurrence of any turbidity on the addition of the sodium chloride is however made It may however be pointed out that the chemical constituents of egg white are not so simple as has been indicated. According to Cameron (1931) it contains at least two different albumins two distinct globulins (all containing glucosamine radicles and the albumins traces of phosphate) and also traces of glucose fats soaps and cholesterol. The ash contains potassium, sodium and chloride in approximately equal amounts with smaller quantities of calcium magnesium phosphate carbonate sulphate silica and iron, with a trace of fluoride

The outstanding facts of the experiments are however quite definite and of especial value in the present connexion as the materials employed were native proteins which had not been subjected to a variety of chemical processes to ensure purity It seems probable that the decrease in viscosity is to be attributed to a discharge of the electrically charged colloidal micellæ and the maximum decrease in viscosity would indicate the point at which all the micellæ were discharged. Since the electric forces of repulsion between similarly charged particles must play a large part in preventing aggregation and consequent coagulation, it might be expected that when all the micellæ were discharged a coagulation and consequent increase in viscosity would set in.

Observations were made upon a sample which had undergone the maximum decrease in viscosity (in this case 32 per cent) during the course of 24 hours after exposure no appreciable recovery or increase in viscosity was observed and after 7 days the sample still showed a decrease of 22 per cent

Fairbrother admits the possibility of the effect being due to chemical changes insufficient to cause alteration in the coagulation point since the only difference observed between control and irradiated specimens was a delay in coagulation in the case of the latter

Taking all facts into consideration, including the work of Crowther upon organo-sols in ethyl and amyl alcohol the balance of probability seems strongly in favour of the physical explanation. In particular is the experiment with λ rays

of value as there is no formation of hydrogen peroxide with its resulting complications, as is the case when water is exposed to β radiations

Oxidation and Reduction Phenomena

The reducing power of β-radiations upon carotin derivatives has been used to show their range of action by means of the butter test Certain samples of butter, when thin walled glass tubes containing radon are inserted into them undergo bleaching for a distance around the tubes corresponding to the range of the β rays A like bleaching occurs if radon tubes are immersed in alcoholic extracts of *e g* carrot and red pepper Colwell (1932) carried out a series of experiments in which the reducing action was still further shown

The experiments were all conducted upon liquid systems by immersion of thin walled glass tubes containing radon. Except where otherwise stated small test-tubes measuring about 4·0 cm in length by about 0 5 cm in diameter were used as the containers The radon tubes were usually of the order of 10 millicuries in strength, were of glass about 2 5 cm. long 0 5 mm. in diameter and with a wall thickness of the order of 0 1 mm thus allowing a large proportion of the β-radiation to reach the liquid in which they were immersed The volume of fluid taken for experiment was about 0·5 c c that quantity being found to fill the test-tube to a convenient height and to correspond more or less with the upper limit of the radon tube when immersed.

During the experiments the mouths of the test-tubes were lightly plugged with cotton wool to exclude dust Except where otherwise mentioned, all proceedings were carried out at the ordinary room temperature and each observation was checked by a suitable control.

Methylene blue. In the earliest experiments (which, indeed, first suggested the possibility of reduction as a result of radiation) a solution of methylene blue (0·1 per cent in H_2O) was taken, and in it was immersed a zinc-copper couple After a day or two the original dark blue colour was nearly discharged. The resulting pale blue liquid on exposure to the air again gradually reassumed the original dark blue colour

as a result of oxidation, while further reduction gave rise to complete bleaching

Such a partially reduced methylene blue solution (0 7o o) was pipetted off into one of the small test-tubes the radon tube (10 mc) immersed the test tube lightly plugged with cotton wool and the whole left for 24 hours When re examined the colour was found to be completely discharged showing further *reduction*

To test the matter further the original (0 1 per cent) methylene-blue solution was diluted (1 part to 9 of H_2O) so as to give a bright sapphire blue when placed in the small test-tubes On similarly exposing this solution to radon of a similar order of concentration to that used in the first experiment (about 10 mc) marked loss of colour occurred in the first 24 hours followed by complete bleaching within a further period of the same duration.

Similar results were obtained with solutions of brilliant green acid fuchsin and safranin in all of which loss of colour is indicative of reduction

Alkaline copper silver and picrate solutions The probability of reduction processes arising as a sequel to β radiation being thus established three of the reagents commonly employed for the detection of reducing sugars—Fehling's solution, ammoniacal silver nitrate and picric acid-caustic-soda mixture were similarly irradiated After the lapse of 24 hours obvious reduction had occurred in the silver solution and the picric acid soda mixture was distinctly reddened

The Fehling's solution showed no apparent change and indeed did not do so when the time of exposure was prolonged to 3 days But at the end of that time the test tube containing it and the radon (together with a control) was placed in an incubator at 35 C The next morning a faint reddish deposit was seen at the bottom of the tube It is probable that the precipitation (not the original reduction) was the result of the heat The control showed no evidence of change

In view of the fact that both ammoniacal silver nitrate and Fehling's solution are notoriously tricky In their reactions the experiments were repeated four or five times Invariably

of value as there is no formation of hydrogen peroxide with its resulting complications as is the case when water is exposed to β radiations

Oxidation and Reduction Phenomena

The reducing power of β-radiations upon carotan derivatives has been used to show their range of action by means of the butter test Certain samples of butter, when thin walled glass tubes containing radon are inserted into them undergo bleach ing for a distance around the tubes corresponding to the range of the β rays A like bleaching occurs if radon tubes are immersed in alcoholic extracts of *e g* carrot and red pepper Colwell (1932) carried out a series of experiments in which the reducing action was still further shown.

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Potassium permanganate. The behaviour of solutions of potassium permanganate under similar conditions was next examined With centinormal $KMnO_4$ solutions gradual decolorization of the liquid with a muddy precipitate of MnO_2 resulted With acid (H_2SO_4) solutions gradual decolorization occurred during which the solution showed a tendency to assume a brown tinge This was due to the presence of a very finely divided brown deposit which gradually sank to the bottom of the tube so that when decolorization was complete there was a small dark brown precipitate. This precipitate was formed when the concentration of the acid was as high as five times normal. On standing it slowly diminished in quantity and in about a week had completely disappeared So far as more or less superficial observations are of any value it appears that the precipitate continues to increase in bulk as long as there is the least tinge of pink in the permanganate When this has been completely discharged solution of the precipitate begins

Experiments with manganous sulphate solutions showed that exposure to β radiation was not attended by any precipitation of MnO_2.

On performing a further experiment with concentrations of N/100 $KMnO_4$ and 5N H_2SO_4 test-tubes of the same calibre (0 5 cm.) but greater length (7 0 cm) were used so as to accommodate 1 0 0 0 of the acid permanganate solution. The radon tube was also increased in length to about 5-0 cm., while its radon content was of the order of 20 mc

The same gradual loss of colour as before was noticed but in addition four or five bubbles of gas were noticed adherent to the outside of the radon tube The brown precipitate gradually formed and within a week after complete decolorization had redissolved

The gas bubbles suggested the presence of traces of hydrogen peroxide in the liquid and, indeed it has long been known that β-radiations act upon water to produce traces of H_2O_2, and also that they decompose it when they act on it in dilute solutions

It is indeed, very probable that in some cases at any rate hydrogen peroxide formation is responsible for the reducing action of the radiations Methylene blue does not contain oxygen and when it is reduced to the leuco-compound the reduction is effected by the addition of hydrogen In the case of the reduction of methylene blue by β rays the first stage is the abstraction of hydrogen from water with the formation of hydrogen peroxide The hydrogen is taken up by the methylene blue which thus acts as hydrogen acceptor and is reduced to the colourless leuco-compound. It may be added that similar bleaching effects were observed with watery solutions of brilliant green and acid fuchsin

FIG 9 Showing effect of β-radiation upon an acid solution of $KMnO$ Oxygen bubbles are seen adherent to the radon tube and the $KMnO_4$ solution loses its bright colour while a precipitate of MnO_2 gradually forms.

To investigate the question of hydrogen peroxide formation 1 c c of distilled water was placed in each of six test tubes (7 0 cm $\times$ 0-5 cm.) and in each was placed a radon tube of the order of 20 0 mc content

After 24 hours the first tube was tested for hydrogen peroxide by adding a drop of a saturated solution of titanic sulphate in concentrated H_2SO_4 On looking down the length of the tube and examining it against a white background a very faint yellow colour could just be detected the control remaining colourless On the five succeeding days one of the remaining tubes was examined, but the tint did not in any case seem deeper than that produced by 24 hours irradiation—i.e the experiment suggested that equilibrium was soon reached when production and decomposition of H_2O_2 were balanced.

Nitrates As a final experiment upon the reducing power

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of β radiations it was decided to ascertain whether nitrates underwent any reduction to nitrites As it seemed probable that a larger quantity of liquid than those used heretofore would be necessary the procedure was modified and an ordinary test-tube was used in which was placed the very thin glass tube containing the radon (about 0 75 cm in diameter and extending nearly the whole length of the test-tube)

The radon content was nearly 50 mc The radon container was placed in the test-tube which was then filled with standard KNO_3 solution (1 c c = 0·0001 g.N) such as is used in water analysis The radon tube was found to be buoyant in the liquid so a slice of cork treated with paraffin wax was carefully slid over the mouth of the containing test-tube to keep it submerged and the cork fixed in place by adhesive labels After 24 hours irradiation the potassium nitrate solution was tested for nitrites by the Griess Ilosvay test (hydrochloric acid naphthylamine hydrochloride and sulphanilic acid) A fine red colour developed thus showing the presence of nitrites the control test being negative

Enzymes

Enzymes or biocatalysts may be defined as catalysts produced by living cells but whose action is independent of the *living cells which produced them.*

For convenience they are divided into two classes according to their usual method of working in the animal body Firstly there are enzymes which normally act apart from the cells which produced them and exert their activity in some definite secretion such are the enzymes secreted into the juices of the digestive tract Secondly there are enzymes which normally exert their effect inside the cells which produce them These are known as intracellular enzymes by appropriate means of disintegrating the cell they can be obtained in the form of extracts and can then under suitable conditions exert their action *in vitro* They are of fundamental importance in bringing about the chemical changes *upon which the life of the organism is dependent* Their outstanding general characteristics are their sensibility to

heat the fact that their action is specific for different substances that they only act within certain temperature limits have an optimum hydrogen ion concentration for working and that in general they act rapidly

Their effects also are commonly such as in their absence would require the use of strong chemical agents at comparatively high temperatures and acting for relatively long periods Further their action is reversible and according to circumstances their effects may be either katabolic or anabolic that is they may either break down complex bodies into simpler ones or they may synthesize complex bodies from simpler units

These characteristics are common to both intracellular and extracellular enzymes but the reversible action is perhaps most prominently shown in the intracellular variety To take a concrete example the net result of the action of the enzymes present in the digestive tract upon proteins is to break them down into their constituent amino-acids These are absorbed by the intestinal epithelium and by the action of intracellular enzymes are again synthesized into proteins The intracellular enzymes present vary according to the chemical constituents of the cells which produce them, and comprehend in their diverse activities the metabolism of all the cell constituents

Enzymes have been named according to the substances upon which they act and are characterized by the suffix *ase* Thus the amylases act upon starch proteases upon protein lipases upon fats and so forth. The substance upon which an enzyme acts is known as the substrate

As is well known their chemical composition is a problem which has occupied the attention of biochemists for many years and in 1926 Sumner reported the isolation of urease[1] in crystalline form The crystals were colourless microscopic octahedra and showed the chemical characters of a globulin Its activity was found to be increased by recrystallization, which was held to be a point in favour of the crystals being the enzyme itself The preparation of these crystals has been effected by subsequent workers but Waldschmidt Leitz (1931)

[1] Urease is an enzyme which acts upon urea, causing the liberation of ammonia.

has shown that they can be hydrolysed by proteolytic enzymes and that the hydrolysed products still retain their activity This seems to indicate that the specific enzyme itself is some specifically active radicle anchored to a carrier which is characterized by high molecular weight but is not necessarily markedly specific in character (Cameron)

It may be added that in 1930 Northup obtained a similar crystalline preparation of pepsin, in which the crystal form was that of microscopic hexahedra

Both Sumner's and Northup's preparations were enormously active and in the case of the urease it was found to be 8 400 times more active than soya bean meal (a very common source of the enzyme) and that at 20° C it could liberate from a solution of urea 120 times its own weight of ammonia in 5 minutes

Thus step by step the ground is gradually being broken though the experiments of Waldschmidt-Leitz would indicate that the protein itself is not necessarily a part of the active enzyme

If the essential chemical constitution of enzymes is as yet an unsolved problem, that of their precise mode of action is no less so it is indeed, probable that the method of action is not the same in all cases

Co-enzymes Reference has already been made to the fact that enzymes generally seem to show a dependence on hydrogen ion concentration for the manifestation of their maximum activity In addition to this it has been shown that for some of them at any rate the presence of other substances is necessary for their working Thus the amylases or starch splitting enzymes of the saliva and pancreatic juice are rendered inactive by dialysis If to such an inactivated, dialysed specimen a small amount of chlorine ions be added the activity is regained In the case of the oxidases the presence of manganese ions is essential for their action. A similar result is obtained by the dialysis of yeast juices by which the zymase (the enzyme which is considered as mainly responsible for the fermentation of sugar) is inactivated but on mixing the residue with the dialysed juice the activity is restored. The nature of this co-enzyme is not yet established

it is however known to be an organic compound, and while some experimental evidence points to the potassium salt of pyruvic acid $CH_3.CO\,COOK$ as the necessary co-enzyme Euler and his collaborators have concluded from diffusion experiments that it is a much more complex organic compound

It is considered by many that enzyme action is intimately associated with adsorption, and that it is essentially a phenomenon of surface activity This would be borne out by the catalytic action of e g certain metal sols in the decomposition of hydrogen peroxide So far as can be ascertained, enzymes themselves are either colloidal in nature or are intimately associated with colloids such as was seen in the crystalline preparations obtained by Sumner and Northup (pp 35–6) If the colloid in question is protein in nature as is suggested by the work of these two authors it is clear from what has been already said that intensive radiation may have an effect in the protein-enzyme complex. As will be seen later radiations have the power of lowering surface tension and also of producing changes in the chemical constitution of some of the proteins In both these cases it is claimed that the result can be produced by the action of doses within the therapeutic limit Incidentally we may revert here to the observations upon the viscosity of irradiated egg white made by Fairbrother (p 27) where the first graph shows that the effect of radiation is not invariably one of steady progress but that a marked irregularity in the process may occur We should not therefore expect the effect to be the same at all stages of the irradiation.

As regards the recorded experimental results of the action of radiations upon enzymes we meet with a marked diversity of statements Considering radium radiations and taking pepsin as an example Bergell and Bickell claimed that its activity was increased Willcock found it was diminished while London stated that radiation was without effect Willcock found destructive effects in the case of trypsin and salivary amylase and Henri and Meyer found destructive effects in the case of emulsin and trypsin Willcock considered that rennin was unaffected, while Schmidt Nielsen recorded a slight inhibiting effect

In all such experiments there is a large number of variable factors to be taken into account The type, purity and concentration of the enzyme extract the presence or absence of certain electrolytes or ions and the hydrogen ion concentration will at once suggest themselves to these must be added the effects of screenage in the case of radium radiations, and more especially the presence or absence of appreciable amounts of β-radiation. It must also be remembered that many enzymes are supposed to be present both in the cells and in solutions as zymogens or precursors of enzymes these themselves are inactive and are in some cases at least more stable in character than the enzymes to which they give rise

In the case of X rays similar discordant results have been noted. Bordier and Galimard concluded that X rays were without effect on peptic digestion. Meyer and Bering found a slight diminution in the activity of peroxidase after one hour s exposure but they also noted that exposure to ultra violet radiation caused complete inactivation after a quarter of an hour According to the same observers autolysis was found to be increased but they regarded this as due not to increased enzyme activity but to the action of the rays upon the nitrogen-containing autolysable constituents of the cell. We shall return to the question of autolysis and give a more detailed discussion of its effects in the next section, but it may be said at once that this seems to be the most probable explanation of the increased autolytic effect

It is not my present purpose to deal further with consideration of this earlier experimental work, but brief reference may be made to the observations of Hussey and Thompson (1923) These workers made careful quantitative studies of the action of radium radiations (β and γ) and of X rays upon solutions of trypsin and pepsin In both cases destructive effects were noted and the experimental results subjected to mathematical analysis

A destructive effect of both β and γ-rays upon the enzyme *succinoxidase was reported by Crabtree (1932)* The enzyme extract was made from ox muscle and exposures were made in circular glass cells 2 cm in diameter These were covered

the action of radiations on living cells On that account a somewhat detailed account of the subject will be necessary

So long ago as 1871 it was noticed by Hoppe-Seyler that dead tissues within the body could undergo liquefaction without putrefaction and he added the observation that the process resembled the action of the digestive ferments (enzymes) It was not until 1891 that it was definitely established by Salkowski that this was a true digestive action brought about by the enzymes within the cells and he isolated from such material two substances then considered as specially characteristic of tryptic digestion—leucin and tyrosin Both of these are of course well known now as being products of protein hydrolysis and both are amino-acids leucin being α amino isocaproic acid and tyrosin β-parahydroxyphenyl α-amino-propionic acid It was however not until 1900 that Jacoby reinvestigated the process of autodigestion and renamed the process autolysis Since that time the subject has been the field of extensive investigation and the resulting amount of literature may fairly be described as enormous. It is believed that in life these intracellular enzymes have a twofold function, anabolic and katabolic but that in moribund or dead cells the katabolic processes predominate so that the end result is the complete destruction and solution of the cell. The various important cell constituents are associated with specific intracellular enzymes so that the enzyme content of different cells is very different Certain enzymes are present in one type of cell and absent from others their presence or absence is found to be intimately related to the presence or absence of specific cell constituents. With the exception of those highly anomalous and specialized cells the red blood-corpuscles enzymes capable of producing autolysis are present in all the cells of the body though in markedly varying degree The absence of autolytic enzymes in the red blood-corpuscles was first stated by Pincussohn and Roques (1914) and further confirmed by Morse in 1921

Details of the various experimental findings are manifestly beyond the scope of the present inquiry but a brief sketch may be useful in order to arrive at a clear idea of the matters to be presently discussed. Firstly there is the group of en

zymes known as proteases which act upon the cell proteins so as to break them down into their constituent amino acids Of these about twenty are known as occurring in different types of protein though present in very different proportions while particular amino-acids are altogether absent in different protein types As regards the nucleoproteins these appear in the first place to be attacked by proteolytic enzymes which attack the protein groups and liberate the nucleic acids These in turn are acted upon by nucleases with the liberation of purine bases which in their turn are decomposed by specific enzymes such as adenase and guanase

It will be seen in the sequel that irradiation has an effect upon autolysis causing in some cases a slight and in at least one other case a very marked, increase Upon which stages of autolysis of either cytoplasm or nucleus the maximum effect is produced is not yet known but as will be shown the early microscopic effects of both autolysis and exposure to β radiation in the cold (0 C) are first seen in the nucleus

Influence of hydrogen ion concentration The degree of acidity or alkalinity has a marked effect upon autolysis Generally speaking a faintly acid reaction (pH = 6) is most favourable but some autolytic enzymes show their maximum effect in a faintly alkaline medium Thus in the liver spleen, pancreas leucocytes and gastric mucosa there have been described pepsin like intracellular enzymes which have an optimum pH = 3 5 that is very definitely acid But in all these tissues there have also been found enzymes of the trypsin type which specially attack peptones or peptides causing their disintegration to amino-acids The optimum pH is about 7 8 In different tissues one or other of these enzyme types appears to be dominant it need hardly be said that variations occur in different species of animal Denby (1918) found that a pH between 5 and 6 was the most effective in the specimens of liver with which he worked He further concluded that the maximum degree of autolysis was reached when both sets of enzymes were able to work together The alkaline-acting enzyme with an optimum pH of 7 8 is not completely inhibited by so markedly acid a reaction as pH = 3

Bradley (1922) confirmed Dernby's view that the first step in autolytic proteolysis is a partial disintegration by an enzyme working between pH = 3 and pH = 7

Dernby (1922) classified autolytic proteases as under

Enzyme	*Substrate*	*Products*	*Optimum reaction*
1 Primary proteases (pepsinases)	Native proteins only	Peptones	Acid
2 Secondary proteases (tryptases)	Denatured proteins and peptones	Peptides or amino-acids	Alkaline or neutral
3 Tertiary proteases (ereptases)	Peptides	Amino-acids	Alkaline or neutral

Autolysis in the liver seems to proceed most energetically at a pH of about 6 or even slightly to the acid side of this Bradley and Taylor (1916) found pH = 6 to be the optimum, while autolysis ceased at pH = 7 4 During the process the reaction of the tissue changed from pH = 7 4 to pH = 6

Lane-Claypon and Schryver (1904) in some early work on liver autolysis found that when the liver was put to autolyse there was a latent period of about 4 hours before autolysis fairly started. This was followed by a rapid rise in disintegration lasting from 4 to 10 hours after which there was a very gradual increase between the tenth and twenty fourth hours The conditions are shown on the accompanying graph where the times are plotted as abscissae and the rise in autolysis as ordinates

Koehler (1923) studied the pH changes occurring in the liver after death the animals being shot The liver brei was found to show pH = 6 6 several hours after death this was not a gradual change the pH changing from normal to 6 7 within a very short time

Koehler Brunquist and Loevenhart (1923) made some observations upon tissue oxidation and reaction which will subsequently be seen to have an important bearing on the problem we are now investigating Decreased oxidation (anoxaemia) was found to produce acidosis and therefore the may-cased oxygen-supply consequent on decreased blood to body will have a marked influence on the change of the

normal pH = 7 4 or thereabouts to the pH = 6 which has been generally considered as the optimum reaction for autolytic enzymes generally

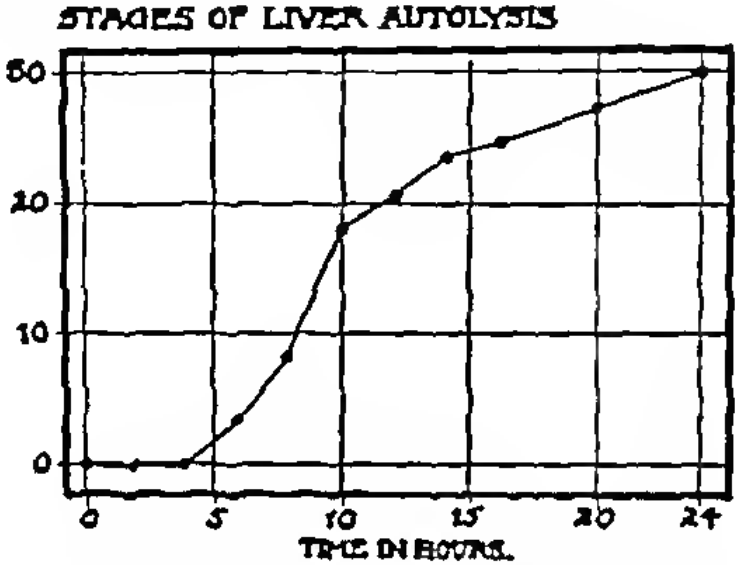


FIG 10 Showing stages in liver autolysis. (Lane-Claypon and Schryver *J Physiol.*, 1904.)

The observations of Sevringhaus Koehler and Bradley (1923) may be summarized as follows

1 Following death the pH increases with almost explosive rapidity the average maximum pH = 6 is reached in from 4 to 48 hours after death after which pH becomes about 6·6 in about 10 days Thereafter a *slow* increase in acidity is noticed.
2 If *acid* is added before autolysis begins the pH diminishes slightly for a few days as hydrolysis proceeds and the bulk of the proteins are converted into amino-acids and *but little change in reaction* occurs afterwards
3 If *alkali* is added before the tissue is set to autolyse the pH may change from 9 to 7 in from 24 to 48 hours
4 The liberation of phosphoric acid accounts for the greater part of the increasing acidity in both the control and the alkaline mixtures
5 The fatty acids produced by autolysis of liver tissue combine with basic groups of the *protein* present and thus raise the pH and increase autolysis
6. The addition of acid to the material which is to be

autolysed causes an increase in the amount of amino acid formed. The optimum point is reached when enough acid has been produced to convert all possible tissue proteins into acid proteins which then form the substrate for autolytic enzymes

By several observers it has been stated that lactic acid is produced in autolysis Working with autolysed muscle Fletcher (1911) was unable to confirm this In other organs however its formation and subsequent destruction is generally believed to occur and its formation or otherwise appears in some degree to depend on the type of antiseptic used in the experiments

The preceding records of experimental work demonstrate clearly that autolytic processes are of extreme complexity The following facts would, however, appear to have been established

1 There is a definite increase in acidity as autolysis proceeds and this acidity favours the general end results of the autolytic processes
2 In the earlier stages the liberation of phosphoric acid serves to establish the optimum acid pH.
3 This is assisted by the formation of fatty acids by intracellular lipases from the fats present in the cell.
4. Lactic acid when produced is again decomposed.
5 Lack of oxygen increases acid formation and therefore stimulates autolysis
6 The state of nutrition of the animal at the time the organ is submitted to autolysis is of the greatest importance since in fasting or starving animals this is much greater than in animals normally fed

These points will be seen to be of essential importance in the consideration of the action of radiations upon cells and tissues

Most of the work upon autolysis has been necessarily carried out by means of chemical analysis at the various stages This involves very accurate complicated and laborious experimental work. It is therefore, of interest to know that attempts have been made both in normal and in irradiated

tissues to correlate histological changes occurring in autolysed tissue with the chemical findings

So far as I am aware the earliest systematic work carried out on these lines was that of Wells (1906) the main object

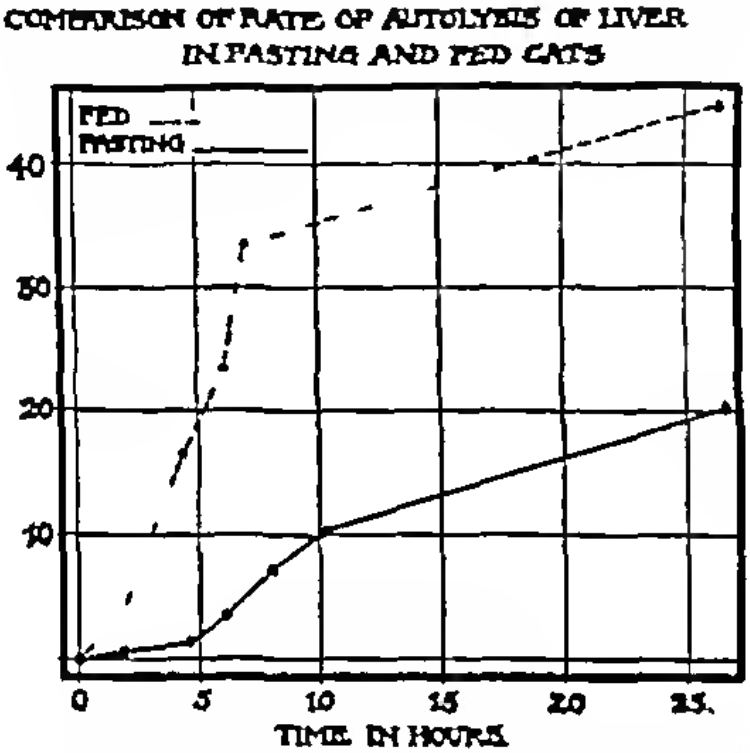


FIG 11 Showing effect of fasting upon liver autolysis. (Lane-Claypon and Schryver *J Physiol.*, 1904.)

of his inquiry was to determine what part autolysis took in the removal of tissue from aseptic necrotic areas such as occur in certain infarcts and as a sequel to the experimental deprivation of blood-supply There are obviously three methods by which dead tissue cells can be removed

1 By heterolysis (Jacoby) or digestion by enzymes secreted by active invading cells
2 By autolysis or digestion by their own intracellular enzymes
3 By the blood-serum

Heterolysis occurs mainly as a result of the action of leucocytes which apparently secrete enzymes capable of digesting not only leucocytic protein but apparently every other sort from serum albumin to catgut ligatures (Wells) Opie has demonstrated the presence of proteolytic enzymes in the

bone marrow which act best in an alkaline medium whereas the antolytic enzymes of the lymphatic glands act most effectually in a slightly acid medium Heterolysis may be either intra or extracellular examples of intracellular heterolysis occur in phagocytosis it is extracellular when the active enzymes are secreted by leucocytes or wandering connective tissue cells Upon the death and dissolution of a cell, the intracellular enzymes are liberated as is evidenced very clearly by the presence of proteolytic enzymes in the urine after extensive superficial burns (Pfeiffer 1914) From pathological evidence it appears probable that the liberation of enzymes from intact normal cells is very slight In the case of severely injured cells the autolytic enzymes act upon the protein and other constituents of the cell itself causing them to break down into simpler bodies

It would seem that the action of serum is comparatively slight this is possibly because the action of any enzymes present is held in check by anti-enzymes present in the serum itself Schryver (1906) found for instance that the addition of serum hindered autolysis of the liver cells and it was also noticed by Opie that the serum of inflammatory exudates has an inhibitory effect upon the action of leucocytic enzymes

Pathological autolysis and heterolysis are mainly brought about by enzymes derived from injured or dead cells In the case of large aseptic infarcts it is found that in the central parts—which are therefore remote from the circulation and from the action of leucocytes or wandering connective tissue cells—the softening and degeneration are brought about by the intracellular enzymes of the cells themselves At the periphery on the other hand extraneous heterolytic enzymes seem to predominate in effecting the removal of the dead tissue

In studying the action of radiations on cells and tissues the question of the part played by autolysis is obviously one of considerable importance Before entering upon a discussion of this matter reference must be made to the work of Corper (1912) in which he endeavoured to correlate the chemical products liberated during autolysis with the contemporaneous histological appearances presented by the

autolysing tissues The paper in which Corper described his experiments and their results is necessarily full of detail and should be studied in its entirety Nevertheless a brief epitome of the results obtained is necessary for our present purpose For his experimental work he selected the spleens of large dogs as providing sufficient material to allow of the necessary chemical analysis

One of the earliest observed histological changes in autolysed material is nuclear pycnosis Dietrich and the Tubingen school attributed this to the loss of water from the nuclear substance Wells is rather inclined to attribute the pycnosis to an intranuclear enzyme whereby the nuclein is attacked with the liberation of nucleic acid. Some facts that will be presently brought forward will be found to give a certain amount of support to this hypothesis the appearance might obviously occur from both causes

Pycnosis is followed by karyorrhexis and karyolysis and the most marked chemical changes occur in the two last phases During the stage of pycnosis almost all the cell constituents are in the coagulable or easily precipitable form much as in the original non autolysed tissue After karyolysis is practically complete the coagulable and precipitable elements gradually decrease in amount but the rate is greatly below that occurring during karyorrhexis and karyolysis Even when microscopic nuclear degeneration is greatly advanced the chemical actions are still in progress though the rate is slow Finally when nuclear structure can no longer be seen

72 per cent of the nuclein N
50 per cent of the original insoluble P
70 per cent of the original insoluble N

are still in the relatively insoluble form—i.e are precipitable and coagulable by alcohol—and two-thirds of the phosphorus is yet in complex organic combination

An attempt was made by Colwell and Gladstone (1932–5) to apply histological methods to the examination of tissues subjected to β-radiation. Radon in thin walled glass tubes was employed for the purpose and the tissue under examination

—rat's liver—was transfixed by the radon tube the whole being kept at 0° C. so as to eliminate as far as possible any autolytic changes in the first instance.

The livers were removed from the animals as soon as they were completely under the anaesthetic[1] and immediately put into ice-cold saline. They were then cut into suitably sized pieces usually about 1 cm. square, and including both the upper and lower peritoneal surfaces of the liver. The portions of liver were then dealt with as the circumstances of the experiment dictated. It will probably add to clearness if the experiments are not detailed in their strict chronological sequence but more or less as the subject has seemed to develop itself when reviewed at the end of the experimental work.

Control specimens[2]. As will be seen later the experimental methods adopted usually necessitated the placing of the tissue in the cold safe at 0° C. for 6 hours. It was therefore essential to determine whether specimens so kept and placed in the cold directly after removal from the body showed any marked differences from a specimen removed from the body and placed at once in Bouin's fluid[3] which was the fixative used throughout these experiments. The differences between these two control specimens were very slight. The nuclei in both were evenly stained and showed a smooth contour but in the specimens kept at 0° C. for 6 hours their appearance was a little more variable than in those placed in the Bouin's fluid while in some cases there was a narrow clear space around the nucleus owing to its contracting away from the surrounding cytoplasm. In these cases the nuclei differed very markedly from those seen in specimens exposed to the

[1] Ether was used as the anaesthetic throughout the present series of experiments.

[2] As will be seen later these controls have an important bearing upon the results, and therefore need fairly detailed consideration.

[3] Bouin's fluid is composed of

Saturated watery solution of picric acid	75 c.c.
Formalin (= 40 per cent. watery solution of formaldehyde)	25 c.c.
Glacial acetic acid	5 c.c.

It is a most excellent fixative for the purpose. Staining throughout was by haematoxylin and eosin.

action of radon, in that they were stained blue instead of the blackish tint seen in the latter. It may be said that preservation at 0° C. for 6 hours had very little effect upon the essential features of the specimens.[1]

Another series of controls was made in which the specimens were transfixed with dummy tubes of exactly the same size as those used for containing the radon in the irradiated specimens. Here there was merely a local pressure effect in the area immediately surrounding the needle-track. The cell outlines and nuclear contour were well defined and even.

Effects of exposure to radon apart from autolysis. In these specimens the portions of liver were removed from the ice-cold saline in which they had been put immediately after removal from the body and transfixed vertically—i.e. so that the radon tube passed more or less vertically through the upper and lower peritoneal surfaces—by the radon tube. The radon tubes were of glass, about 1·5 cm. in length and 1 mm. in diameter, with a wall thickness of about 0·08 mm. The radon content of the tubes was, except when otherwise specified, approximately 10 mc. Exposure was made for 24 hours, the specimens being kept at 0° C. throughout and then transferred to Bouin's fluid.

The appearances then seen were in marked contrast to the controls. The area immediately around the needle-track was deeply and diffusely stained with eosin, and the intercellular divisional planes were markedly indistinct or had entirely disappeared. (See p. 70.) The cells thus seem to be fused with one another so as to form continuous trabeculae which are arranged concentrically round the perforation produced by the needle. The nuclei of the liver cells in the zone immediately round the perforation, where they were exposed to the most intensive β-radiation, were very deeply stained and appeared almost black, the details of nuclear structure being

[1] Some experiments were also carried out in which the specimens were placed in ice-cold 50 per cent. alcohol, then transferred through gradually increasing strengths to absolute alcohol. The specimens were kept in the ice-chest at 0° C. throughout the progress from 50 per cent. to absolute alcohol. The idea was to eliminate possible changes due to treatment by strong electrolytes. These specimens showed no differences from the Bouin fixed specimens, except that the fixation was not quite so good.

obscured or quite invisible In many cases the nuclei were flattened or elliptical in outline conformably to the flattening of the cells in which they lie As in the control this flattening is the result of the mechanical pressure caused by the needle In some places detached fragments of chromatin could be observed and were apparently due to breaking up of some of the nuclei The endothelial lining of the hepatic sinuses showed some disintegration, and the shrunken highly pycnotic nuclei had in some cases escaped from the endothelial cells

The cytoplasm of the hepatic cells in the zone around the needle track was deeply stained with eosin, the coloration being markedly diffuse whereas in the control specimens it was limited to the granules and cytoreticulum the hyaloplasm being in this case either very lightly stained or unstained This diffuse coloration of the cytoplasm appeared to be due to the breaking down of the secreting granules and the diffusion of their contents in the matrix of the cell body

The zone of most intense action around the needle-track measured about two-thirds of the diameter of a liver lobule Beyond this area were outlying patches of liver tissue which showed the more marked effects just described but these were smaller and the changes less marked with increased distance from the needle-track Elsewhere in this intermediate zone the outlines of the liver cells were more distinct, and the granules more normal in appearance The distribution of the granules and their size were however observed to be very variable the sharp contrast between the clearly outlined well-stained granules and the clear cytoplasm of the control specimens was lacking in the irradiated specimens The cytoplasm itself showed a peculiar ground-glass like appearance in the irradiated specimens as if a very fine precipitate had been produced this was in marked contrast to the bright and clear appearance of the cytoplasm in the controls As with the nuclear changes this was most marked in the region of most intensive β-radiation.

Effect of autolysis without preliminary irradiation In the series now under consideration portions of liver were kept in the cold (0 C) for 24 hours so that they had exactly the

same conditions—except for radiation—as those in the preceding series Autolysis was allowed to progress for two hours at 35 C in chloroform saline after which the tissue was removed at once into Bouin's fluid Staining as before was with haematoxylin and eosin.

To the naked eye the section appeared evenly stained but examination with a low power ($\frac{2}{3}$ inch) objective showed that the staining was patchy in distribution In some areas the cytoplasm had broken down and clear unstained spaces were present in it in other situations the granules and cytoreticulum showed deep staining with eosin giving the impression that the autolysis had been more active in some places than in others On the other hand in those areas where the cytoplasm had broken down the cell boundaries and cytoreticulum were often abnormally distinct owing to the disappearance of the granules and the replacement of the matrix by clear spaces Nothing was left of the cell body in these situations but the reticulum and the cell membrane

The nuclei of the liver cells in the central and intermediate parts of the section varied considerably in size some being contracted while others were larger than normal sometimes a clear zone was present around the nucleus

Effect of autolysis with preliminary radiation The specimen was exposed to radon for 24 hours at 0 C and then allowed to autolyse for 2 hours The effects in the zone immediately around the needle track were of much the same character as those observed in radiation without autolysis but were much more marked The cells were less deeply stained with eosin and showed the same kind of fusion owing to disappearance of the cell membrane The changes observed were more marked also than in the specimens submitted to autolysis alone and were also best seen in the zone of most intense irradiation.

The effect of previous irradiation is therefore that the autolytic changes which occur after exposure are more marked than in non irradiated specimens

Effect of exposure to liquid air before autolysis These experiments were undertaken in order to observe the effects of an agent which it was thought would probably cause severe

damage to the cell substance without inhibiting the subsequent action of the autolytic enzymes

The effects of exposure to liquid air alone were first determined The freshly removed liver was as before placed

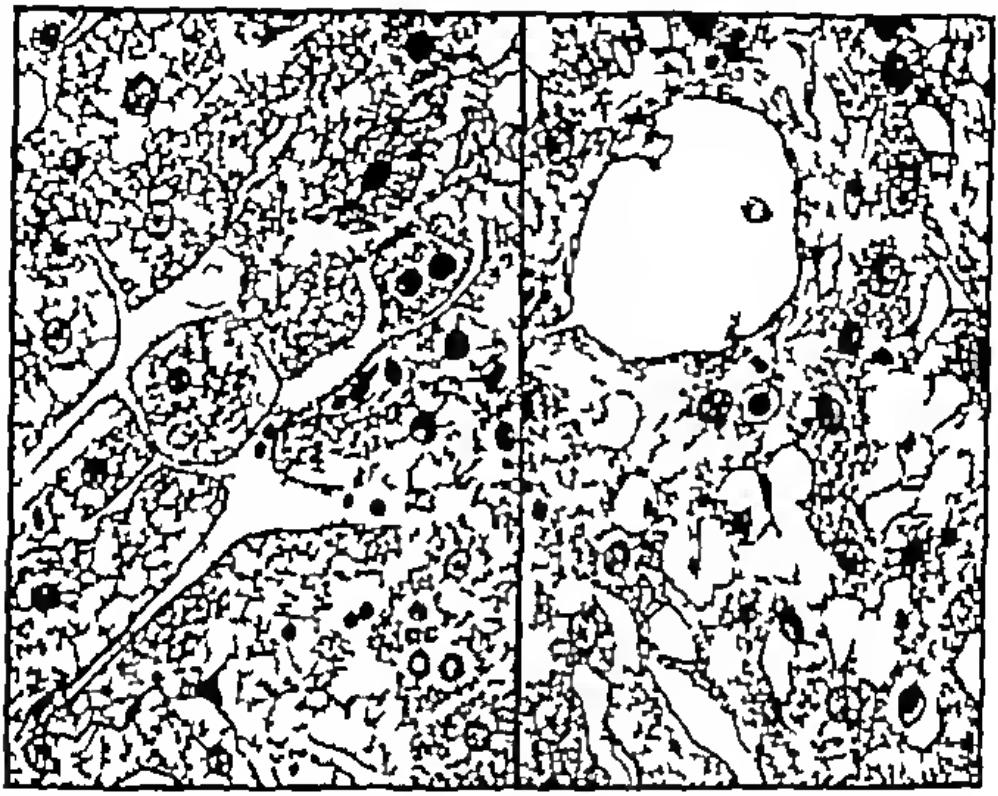


FIG 12. Sections of rat liver (A) Autolysed without irradiation. (B) Autolysed after irradiation by β rays: the large space in the figure indicates the track of the glass tube containing radon.

immediately in ice-cold saline after which it was dropped into a vessel containing liquid air (temperature = −150 C) After evaporation of the liquid air and when the specimen had again become softened, it was placed in ice-cold *50 per cent* alcohol fixation being completed in different strengths of alcohol up to absolute alcohol. Fixation throughout was *carried on in the ice-chest*

The most striking change noticed in the sections was a great increase in the size of the intercellular and intertrabecular spaces There was also a breaking down of the matrix of the cell bodies with the formation of intracellular spaces in the cytoplasm The nuclei of the liver cells also showed variations in size and depth of staining some of them being

remarkably small The appearances throughout were consistent with what might have been expected from the physical changes to which the material had been subjected Some of the pieces of liver after removal from the liquid air showed marked fissures and of course were so hard when first removed, as to be easily broken to pieces by a blow from a hammer

When the specimens were removed from liquid air and then allowed to autolyse for 2 hours the results were very different After autolysis they were found to be so diffluent that they could not easily be moved, and it was necessary to pipette off the saline solution in which they had been allowed to autolyse and to pour the fixing fluid over them There was found marked destruction of tissue throughout the whole specimen the nuclei of the liver cells were pycnotic and so reduced in size that the diameter was not greater than that of a red blood-corpuscle The cytoplasm generally was ill stained and the endothelial lining of the hepatic sinuses was completely destroyed The autolytic changes were seen to be greatly exaggerated when compared with the appearances seen in normal liver allowed to autolyse for the same period. Severe damage to the cells such as is caused by exposure to liquid air thus increases autolysis and the extreme nuclear pycnosis is very marked.

It is interesting that the earliest noticeable effects of both autolysis and exposure to β-radiations in the cold (0 C) should be the production of nuclear pycnosis.[1] The matter undoubtedly calls for further investigation but since one well known effect of radiation is upon the nuclear membrane the pycnotic appearance in this case may be due to loss of water in the early stages of autolysis as is suggested by Dietrich and his collaborators Equally of course this may be the effect of direct action of the radiation upon nuclear structures and indeed the peculiar and characteristic blackish coloration of the nuclei when stained with haematoxylin suggests that direct chemical action upon nuclear material is likely to be at any rate a contributory factor

[1] Distinct evidence of the beginning of nuclear pycnosis can be seen after one hour's radiation in the cold.

So far as the liver is concerned it would appear that the increased autolysis due to irradiation under the circumstances described is slight or moderate and it may be said that in all cases the livers used were those of well fed non fasting rats

With the intestinal mucosa the effect of radiation upon subsequent autolysis has been shown by Warren and Whipple to be very marked It is well known of course that the mucous membrane of the small intestine is exceedingly sensitive to irradiation when irradiated mucosa is removed and allowed to autolyse comparison with the autolysis of control specimens shows a striking increase The differences between irradiated and non irradiated autolysed and non autolysed specimens are exceedingly well seen in microscopical preparations

Probably comparative examinations upon the effect of radiations on autolysis in different tissues both normal and neoplastic would reveal considerable differences in the effects produced. Moreover in living tissues a number of other factors which are directly affected by radiation necessarily come into play Differences in oxidation and nutrition dependent on altered blood supply will at once suggest themselves and indeed, these secondary effects may be of considerable importance in determining the progress of autolysis in cells which have already been injured by exposure to radiation.

As already said increased autolysis as a sequel to radiation is most probably not due to any activation of the enzyme but to a damaging effect of the rays upon the tissue elements which form the substrate The matter will be referred to subsequently when some of the actions of radiations upon neoplasms are under consideration.

Cell Lipides

The Lecithins and Cholesterol

The term lipoids was long used to designate a heterogeneous collection of substances which were more or less connected rather by their physical than by their chemical properties All the substances so called are insoluble in water

but can be extracted by such fat solvents as alcohol ether and chloroform The term has been so loosely employed that its disuse seems advisable a more comprehensive term *lipide* has been suggested to include such substances as those mentioned below Many attempts at classification have been made but perhaps the following scheme of classification[1] for fats and bodies with similar physical properties is as convenient as any It will be seen that upon this view the fats themselves are included as a sub-group of the lipides which as a whole are characterized by the physical properties already mentioned.

1 *Simple lipides* (esters of fatty acids with alcohols)
  A Fats (oils) All glycerides
  B Waxes Esters of fatty acids with higher alcohol.
  C Cholesterol esters
2 *Complex or compound lipides* (compounds of fatty acids with alcohols but also containing other radicles)
  D Phospholipides or phosphotides Lecithins cephalins sphingomyelins
  E Glucolipides (contain carbohydrate radicles and nitrogen but no phosphorus) cerebrosides
  F Aminolipides Sulpholipides &c
3 *Derived lipides* (compounds derived from the above groups by hydrolysis but which still possess general lipide characters)
  G Saturated and unsaturated higher fatty acids
  H. Higher monatomic alcohols including the sterols Cholesterol.

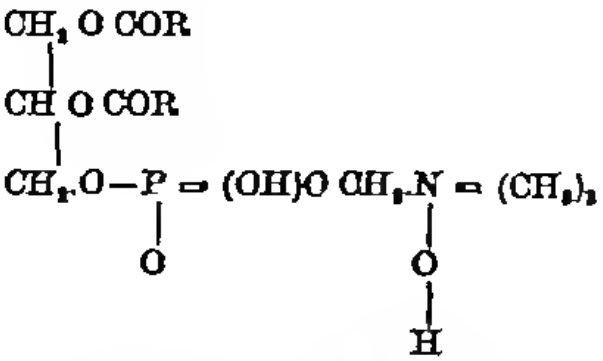
The Lecithins The lecithins belong to the second group in the preceding classification—complex lipides Upon hydrolysis they yield glycerol phosphoric acid choline and fatty acids All lecithins are dextrorotatory and as the derived glycero-phosphoric acid is also active the following formula has been assigned to it

$$\begin{array}{l} CH_2.OH \\ | \\ CH.OH \\ | \\ CH_2.O.PO.(OH)_2 \end{array}$$

[1] As given by Cameron.

so that the phosphoric acid group is not united to the middle carbon atom of the glycerol chain

The general formula for the lecithins themselves may be written



It has from time to time been suggested that X ray and radioactive radiations have an almost specific destructive effect upon lecithin causing its decomposition and that the free choline (trimethylammonium hydroxide) thus liberated is the toxic agent in all and sundry varieties of cell destruction due to radiation.

All recent work has gone to negative such a view and irradiation of lecithin by intensive doses of X rays has repeatedly been found not to cause liberation of choline Personally, I have exposed to X rays samples of as pure lecithin as I could prepare from egg yolk for 24 hours and obtained no evidence of any choline liberation The preparations were placed in small glass dishes about 1½ inches in diameter and covered with thin mica sealed with paraffin wax.

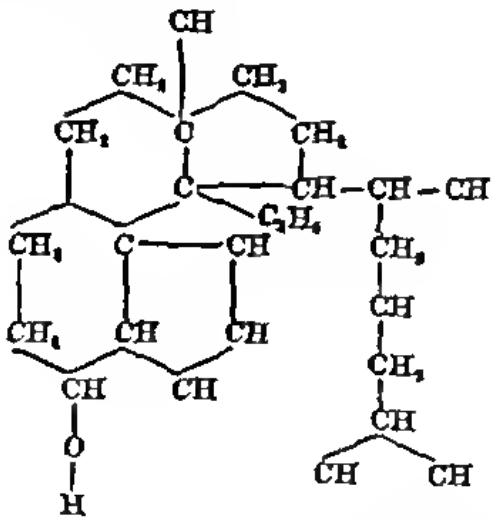
As this groundless theory of the action of radiations continues from time to time to make its appearance it has been necessary to refer to it because experimental evidence does point to an action of radiation upon lecithin though not of the crude nature previously suggested. Lecithin is closely associated with cholesterol in cell and nuclear membranes and this is certainly the case in the cell membrane of red blood-corpuscles In these conditions the lipides are associated and linked with protein groups. Radiations are believed to act upon these protein lipide complexes so as to disrupt them

Cholesterol

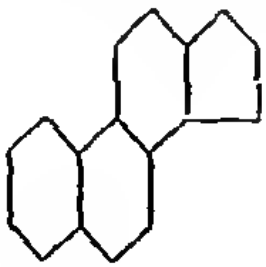
Both cholesterol and lecithin have been credited with playing an important part in the biological actions of radia

tions and it has even been suggested that decomposition products formed under these conditions are the fundamental sources of the lethal action of radium and X rays upon animal cells Further research has shown that this somewhat crude hypothesis is almost certainly incorrect but that a secondary action takes place in which protein lipide com plexes are disturbed or destroyed, seems very probable and is supported by so good an observer as Fernau

Of the mode of formation of cholesterol in the body we are still in the dark the view that the reticulo-endothelial system is largely responsible for its formation appears to be gaining ground Its exact chemical composition is yet under discus sion but that it has carbo-cyclic units a secondary alcoholic *group and one unsaturated linkage are established facts* Windaus considers the following formula as most probably representing its composition



It has now been established by Rosenheim and King that the nucleus of all the bile acids and sterols including cholesterol is



The empirical formula of cholesterol is $C_{27}H_{44}O$

What is certain is that it is extremely widely distributed in the animal kingdom and in a careful study by Dorée (1909) of metazoal forms from the Chordata to the Cœlenterata it was found in all cells of all the animals examined with the single exception of the starfish (*Asterias rubens*) Its presence in all cells of the higher animals may be taken as established, and their ubiquity has led to the conclusion that it must have important functions Pathologically it is found to be accumulated, often in its characteristic crystalline form in tissues where cells are undergoing slow destruction and where absorption is poor it is also a constituent of all pathological exudates It is stated to be unaltered during the process of autolysis

There are two ways in which it is likely that cholesterol is of importance in the animal organism Firstly as a component of protein lipin complexes as in cell and nuclear membranes and, secondly as a factor in immunity reactions and vitamin formation The relation of cholesterol to certain hormones and to the active agent in carcinogenic tars has been summarized by Dodds (1934) in his Goulstonian Lectures It is of interest that the addition of cholesterol to the red-cell suspension retards or inhibits the haemolytic action of such agents as saponin it should however be noted that this property is not shared by the lipins The importance of lipins in the various processes of immunity is recognized and it has been claimed though it is not generally agreed that lipin extracts may replace the actual red blood-cells as the antigen in the preparation of haemolytic sera

Considering the general character of the various cell constituents there is a probability that the lipin elements are present in colloidal form and may therefore be concerned in surface actions It has been thought that the adrenals are at least an important source of some of the body lipides the fact is known that 33 6 per cent. of the human adrenal consists of ether-soluble materials (Wells 1908) of which 20·6 per cent. are cholesterol while 33 per cent are lecithin.

This is a brief summary of certain of the known and supposed functions of cholesterol in the animal economy It is

known along with lecithin to form a part of the cell membrane of the red blood-corpuscles it may well be combined with protein and the highly specialized function of the cell membrane of the red blood-corpuscles is well known

Cholesterol and closely allied bodies are known under certain conditions to be important in vitamin production, and in this respect the possibility of an inhibiting action of X rays and radium has been suggested.

The decomposition effect observed by Roffo and Correa when solutions of cholesterol in chloroform are irradiated has been found to be due to a secondary action caused by the action of the radiation on the solvent When cholesterol is dissolved in solvents which do not contain chlorine—benzene ether alcohol—the typical colour reaction with acetic anhydride and sulphuric acid is neither destroyed nor diminished The effect may not be manifested, even in chloroform solutions of the strength used by Roffo and Correa (0 02 per cent) I have exposed such solutions enclosed in quartz tubes sealed with corks soaked in paraffin wax, for several hours to X rays with no obvious effect on the typical colour reaction and similarly exposure to 30 mgm radium element screened with 0 5 mm platinum for 4 days was found to be without effect In this case I may say that the chloroform used was the purest anaesthetic chloroform specially obtained for the experiments in hand and that the solutions were protected from light throughout the experiments

Roffo and Degiorgi (1929) by irradiating blood-serum *in vitro* claim that a destruction of cholesterol does occur and that the amount of destruction depends upon the duration and intensity of the radiation They further state that the destructive effect is greater when the original cholesterol content is small.

During the course of some experiments which are still (February 1935) in progress a certain amount of evidence has been forthcoming that cholesterol undergoes *oxidation* as a result of exposure to β-radiation. It is indeed well known that it readily and even spontaneously undergoes oxidation and that the reaction is irreversible

The lecithins on the other hand if they contain any

unsaturated fatty acid groups tend to undergo *reduction* Hence if cholesterol and lecithin form an essential part of cell membranes as is generally supposed, it is clear that exposure to radiation may alter their chemical composition in a very direct manner and so affect their permeability As will be seen later the histological changes in both cell and nuclear membranes occurring after radiation are very marked (see Chap III)

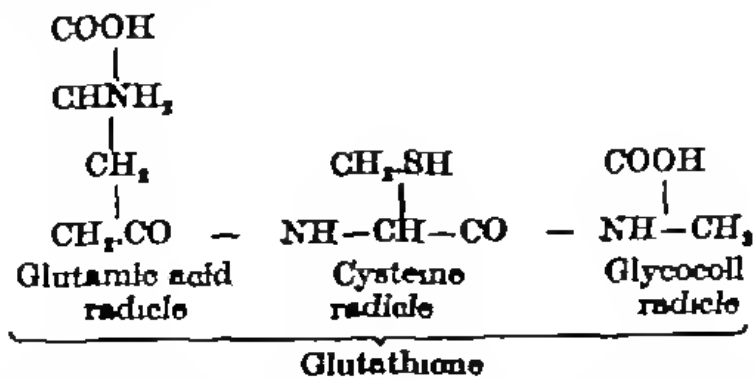
Glutathione

This substance which is of fundamental importance in cell oxidations was first isolated from muscle by Hopkins (1921) It is not present in blood plasma but occurs in varying amounts in all the body tissues examples of the glutathione content of some tissues are

Striated muscle	0·06 per cent.
Cardiac muscle	0·12 ,,
Non-striated muscle	0·13 ,,
Liver	0·38 ,,

It is believed to be present in larger amounts in foetal than in adult tissues and especially abundant in cells of the so called embryonic type where it acts as a powerful stimulant to mitosis

Chemically it has been found to have a peptide structure being formed by the combination of glutamic acid cysteine and glycocoll radicles according to the following scheme



It may be regarded for the sake of simplicity as a hydrogen *sulphide molecule* in which one of the hydrogen atoms is replaced by the glutathione radicle If this is expressed by

the abbreviation G the oxidation of reduced glutathione will be represented by the accompanying formula

$$2(G.SH) \leftrightharpoons GS.SG + 2H$$

In living cells its part is that of a transporter of hydrogen and, as indicated above the reaction is a reversible one It appears also that there is present in the tissue cells a substance of which the chemical nature is as yet undetermined It is like glutathione thermostable but differs in being insoluble in water This substance contains hydrogen atoms with which it parts readily Indicating this body by the formula XH_2 its reaction with oxidized glutathione may be represented by the following equation

$$XH_2 + GS.SG \leftrightharpoons X + 2(G.SH)$$

The reduced glutathione thus formed can part with its hydrogen to any suitable acceptor in the cell this is most probably oxygen and the compound resulting from the union of the two elements hydrogen peroxide Hydrogen peroxide when acted upon by the intracellular peroxidases can in time part with its oxygen which is then available for purposes of cell metabolism A certain amount of evidence has been brought forward to show that glutathione itself may exist in the form of a complex organic peroxide and so act as a transporter of oxygen.

Its importance in processes of cell oxidation has been amply verified by experiment Muscle tissue for instance if deprived of its glutathione by extraction with water ceases to consume oxygen when removed from the body If to such washed and glutathione-free muscle tissue glutathione is added oxidative processes recommence with the accompanying consumption of oxygen.

Coldwater (1930) experimenting with the planarians *Planaria agilis* and *Proctyla fluviatilis* in which the glutathione content is normally high found that it was markedly diminished by exposure to large doses of X rays (2000 R) The probability of the great clinical importance of this experimental work when the therapeutic radiation of neoplasms is concerned is evident since as already said glutathione is present in unusually large amounts in cells of the embryonic

type in which it is believed to act as a powerful stimulus to mitosis any agent tending either to its destruction or to inhibition of its action will clearly have a marked effect upon the oxidative processes and therefore upon the vitality of the cell

Oxidation and Carbohydrate Metabolism of Tissues

Mention may suitably be made here of the work published by Crabtree (1932) on the effects of radium radiations upon tissue oxidations and carbohydrate metabolism There are as he points out many practical difficulties to be over come in such investigations for instance the tissue examined must be such that it will survive when removed from the body for a sufficient time to enable the necessary readings to be made The latent period which manifests itself in a number of histological examinations of tissue after irradiation must also be taken into account in these experiments If tumour tissue is the subject of investigation care must be taken to ensure the homogeneity of the specimen as otherwise totally fallacious results might be obtained areas of necrosis or of partial degeneration would be specially liable to vitiate the results of the experiment In addition there is the very interesting fact that specimens taken from tumours histologically identical in every respect may normally give very different results when the phenomena of their metabolism are the subject of inquiry

Crabtree used two radium applicators each containing approximately 58 mgm of radium element and screened with 0·12 mm of silver In the *in vitro* experiments there was in addition a layer of glass and a layer of liquid (Ringer's solution) The preparation of the Ringer's solution is a matter of importance and special measures are required to obtain a pH of 7 46 at the beginning of the experiment The tissues which were found most suitable were the normal testis and spleen of the rat and Jensen's rat sarcoma

For the experiments performed on irradiation *in vitro* the apparatus shown in the figure was used. In the case of Jensen's rat sarcoma the actual quantitative results in a series of fifty cases showed variations but the general quali

tative result was the same— a progressive deterioration of the respiratory power with no concomitant change in aerobic glycolysis until the respiration has become negligible The experiments were carried out for varying periods generally from 10 to 12 hours, though some were extended to 24 hours

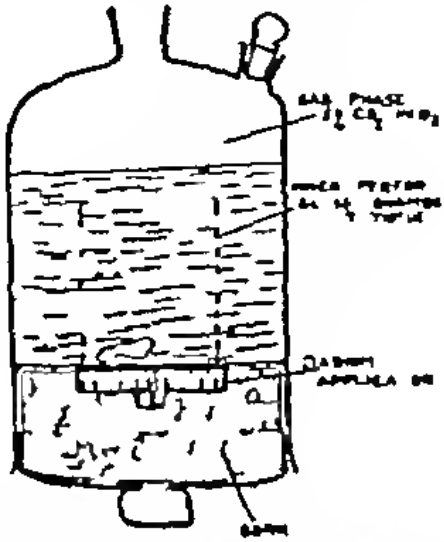


FIG 13 Apparatus for determining the influence of radiations upon oxidation and carbohydrate metabolism. (Crabtree *10th Report Imp Cancer Research Fund* 193)

It is noteworthy that the same progressive deterioration of the respiratory function occurred in non irradiated tissues but much more slowly Irradiation had not initiated but only accelerated a normal process in tissues removed from the body

Allusion has already been made to differences in the behaviour of sections of tumours which were to all appearances identical in histological character three instances of this may be cited

1 This specimen showed a marked fall in respiratory function quite suddenly after about 3½ hours irradiation By the tenth hour this had fallen almost to zero
2 In a second specimen which was an example of the most commonly occurring type there was a gradual fall manifesting itself distinctly in about 4–5 hours and with a gradual fall afterwards
3 In a third example change was but slight up to 8 hours and a marked fall began about the tenth hour

Effects of similar character were noticed with normal rat

testis and spleen the characteristic features were a latent period followed by a fall in the respiratory process during which the aerobic glycolytic function seemed practically unaltered

Crabtree's general summary is as follows

The respiratory processes are always affected

(*a*) *In vitro* tissues under constant irradiation gradually lose their power of using oxygen after an initial period of some hours, during which no change is detectable. Control tissues meanwhile respire freely though ultimately they suffer the same fate. Radium radiation effects an acceleration of a naturally occurring degenerative process.

Glycolytic processes are much more resistant to the damaging influence of radium radiation. During the period of progressive decay of the respiratory power to a negligible value glycolysis proceeds unimpaired

(*b*) *In vivo* similar effects are produced though less rapidly

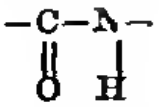
The delayed effect of radium radiation on the Jensen rat sarcoma leads to a fall in the magnitude of the respiration, or a damaging of its effectiveness in checking glycolysis, which results in the appearance of an abnormally high glycolysis

On rat testis and spleen a fall in respiration is brought about without any corresponding rise of aerobic glycolysis.

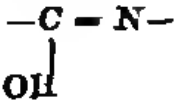
No differential action of the effects of radium radiation could be established between normal and tumour tissues while tumour tissue of apparently exactly the same structure showed qualitative variations among themselves

Effects of Radiation upon the Chemical Composition of Proteins

Some account of effects of the action of radiations upon proteins considered as colloidal systems has already been given There are also however certain changes set up in the chemical molecule whereby the proteins are rendered more acid in character The general consensus of opinion seems to be that this is due to a change in certain polypeptide linkages whereby the group



is transformed into



The sensitiveness of different proteins to X rays in this respect is found to vary

The influence of radiation upon the gold numbers of different proteins would appear to vary that of globulins was found by Mond to be unaltered, while that of albumins was slightly raised.

Colwell and Russ (1911) endeavoured by a quantitative application of the biuret reaction to ascertain if any decomposition was produced by X rays in solutions of Witte peptone The results were in all cases negative Recently (1933) the experiment of trying the effect of intensive β-radiation upon Witte peptone solution was made by Colwell. A 2 per cent solution of Witte peptone was prepared, and about 5 c c placed in a small test tube with a few drops of toluene In this was placed a thin sealed glass tube containing 210 9 millicuries of radon. At the same time a control was put up without any radon. Both tubes were kept at room temperature for a week, after which samples were removed and tested.

The observations were carried out in Nessler glasses 50 c c of NaOH solution 0-5 c c of 1 per cent $CuSO_4$ and 1 c c of the peptone solutions being taken It was found that the irradiated sample was decidedly paler than the control. A further test was made using ammonium hydrate instead of sodium hydrate The less powerfully ionized alkali only appears to attack the more hydrolysed constituents in the Witte peptone and care must be taken that too much copper is not added as then the pink colour due to the biuret reaction is masked by the deep blue colour produced when a copper salt is added to ammonia On trying the experiment with the irradiated and control samples of Witte peptone the results were seen much more definitely than when the ordinary $NaOH+CuSO_4$ reagent was used. Only a small amount of the irradiated material was available for tests but speaking broadly it seemed necessary to use about twice as much of the irradiated as of the control specimen to produce the same depth of tint

Of course the amount of β-radiation used was enormous and no inference can be drawn that therapeutic irradiation

would have any disintegrating effect on the native proteins of living cells The experiment is however suggestive as indicating that a sufficient intensity of β-radiation can still further split up some of the constituents of Witte peptone It further suggests that experimental work upon the action of radiations on some of the synthetic polypeptides might indicate points in the various amino acid linkages which are most susceptible to the action of radiations

From the foregoing account it will be seen that most of the cell constituents are affected physically or chemically or both by exposure to X rays or the β and γ-rays of radium. The effects produced by X and γ-rays are generally much less striking than those produced by β-radiation, but so far as can be ascertained though less in degree they are the same in character Moreover physical changes in proteins such as alterations in viscosity and diminution of surface tension, have been shown by Wels and Thiele to occur with doses of X radiation well within the limits of those used in therapy Oxidation processes have been shown to be modified both by the direct action of the radiations and by their action upon oxidizing enzymes

Although the chemical effect upon lecithin and cholesterol does not appear marked it is nevertheless believed that radiation does affect protein lipide complexes and this may partly account for the changes which will subsequently be described as occurring in the nuclear and cell membranes. Indirectly it will also lead to disturbances in the electrolyte equilibrium of the cell and therefore in the various buffer systems which play so large a part in cell metabolism

CHAPTER III

GENERAL EFFECTS OF RADIATION ON CELLS

Having given a brief consideration to the essential features of normal cell structure and to some of the chief chemical effects of radiation which appear to have a bearing upon the action of radiations on living tissues we must next proceed to the review of certain general biological findings in the case of irradiated cells

For this purpose two distinct classes of material are available Firstly there are fixed and stained specimens and secondly there are cells examined in the living state usually in the form of tissue cultures Though it is perhaps rather the fashion at the present time to under-estimate the value of the former the tendency is nevertheless one which I cannot help regarding as a mistake Valuable information can still be obtained from preparations made by the older methods especially when the findings are correlated with the results obtained by examination of tissue cultures For some purposes fixing and staining are essential, and they have one obvious advantage over the newer method namely that they permit of the examination of whole organs or of embryos at various stages of development Above all, the specimens so obtained are permanent but at the same time adequate controls and the most careful scrutiny with a high power are of paramount importance

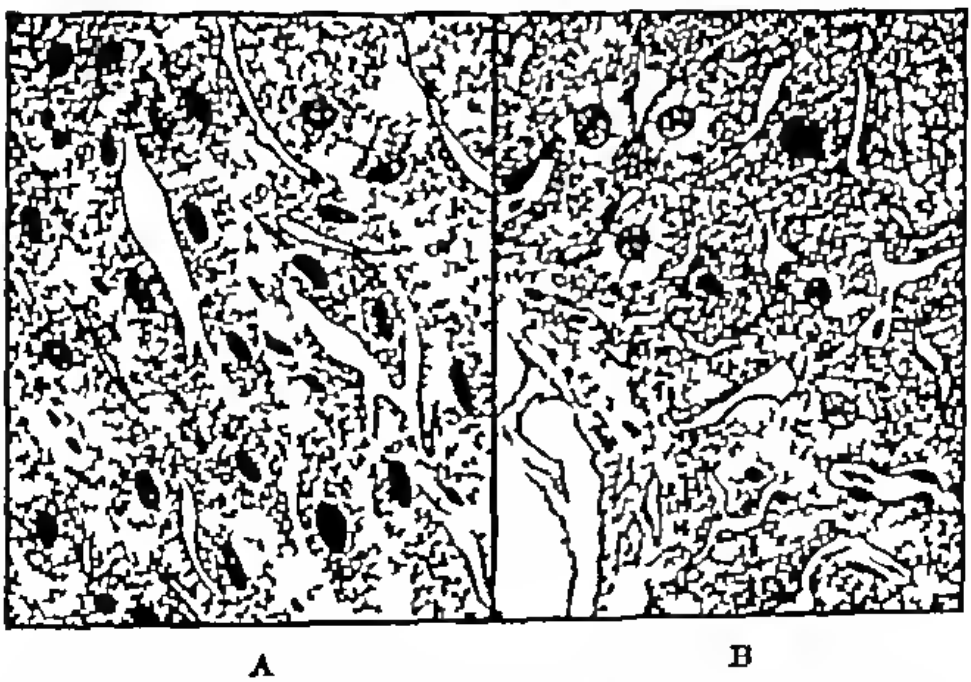
We shall therefore in the following account take into consideration the effects of radiation as manifested in specimens of both classes it will perhaps add to simplicity of explanation if the various units of cell structure are considered in the same order as they were previously enumerated and briefly described

The Cell Membrane

Generally speaking references to the effects of radiation upon this most important structure are comparatively few Most of the earlier investigations were directed to research

of the exposure this can hardly be due to autolysis In control specimens transfixed with dummy glass tubes similar to those used for containing the radon, these appearances were absent.

FIG 15. Normal rat liver Cell outlines distinct. (Gladstone)



A B

FIG 16. Irradiated rat liver (radon, 10 mc) (A) Near radon tube Cells showing pressure effects and loss of cell outlines; (B) From same specimen as preceding, but farther from radon tube. Cell outlines indistinct or lost (Gladstone)

At the least it would appear then that the effect of exposure to either X rays or β radiation has been such as to produce some alteration in the cell membrane which causes definite and consistent changes in the fixed and stained specimens

This view is strongly supported by some experiments recorded by Colwell and Thomson (1927) The skin of frog tadpoles was selected as the material for experiment The

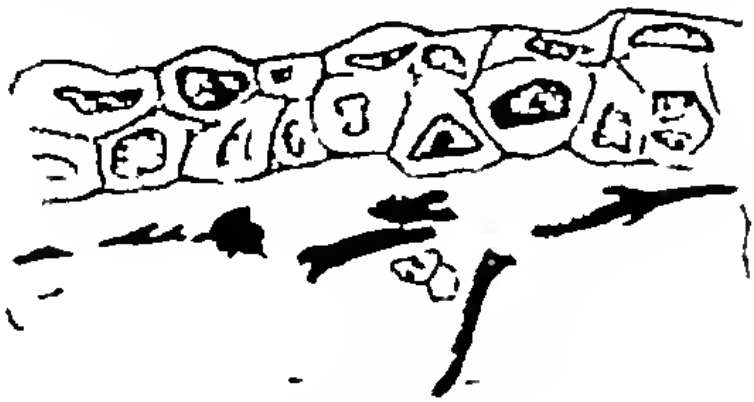


FIG 1 Normal tadpole skin. Cell outlines well marked

tadpoles were exposed to the action of X rays in shallow dishes of water in some cases hydrosols of different elements were added to the water with the idea that the effects of secondary radiation might also be observed

For these purposes the skin of the tadpole is particularly suitable since the epidermis consists of only two layers and the cells are so well marked off from one another as almost to suggest the existence of a cell wall

The source of radiation throughout the experiments was an ordinary medium focus Coolidge tube excited by an induction coil working at 2 milliamperes with a seven inch spark gap the focal distance from the surface of the water was 6½ inches

Exposures were made for 1½ hours 2 hours and 5 hours while after radiation some specimens were killed at once others being allowed to survive for different periods varying from 1 to 7 days The appearances obtained upon microscopical examination of the epidermis varied with the length of exposure with the presence or absence of colloids and with

the time of survival In all the irradiated specimens the outstanding changes were in the main atrophic or hyper trophic In the accompanying figures Fig 17 shows the normal epidermis with the cells distinctly marked off from one another The next figure (18) shows the skin of a specimen irradiated for 1½ hours and allowed to survive for 24 hours.

FIG 18 Irradiated tadpole skin. Loss of distinction of cell outlines.

The loss of cell outlines is very marked and already there may be seen the beginning of that syncytium formation which is so characteristic of specimens which show marked hyperplastic changes The specimen is of interest as showing that the appearance of a syncytium is due not only to nuclear proliferation but to elimination of cell outlines The irregu larly shaped black objects lying in the corium are pigment cells Their fragmentation after exposure to X rays is usually a very marked and characteristic feature and it may fairly be argued that since fragmentation implies a solution of continuity of the limiting membrane, this has also been affected by the radiation.

When the radiation was carried out in the presence of different colloids the main results varied With copper the effect was destructive here also loss of cell outline was a striking feature With silver the initial changes were hyper trophic as shown in Fig 19 it will be noticed that the hyperplastic epithelium shows no distinction of cell outline and that the nuclei are embedded in a more or less homo geneous syncytium with no individual cell differentiation

Admittedly the dose of radiation was far beyond what

would be given therapeutically with the type of apparatus employed. The result does however indicate the direction in which the effect of X rays tends A further point to be borne in mind is that when the cell boundaries are so altered as to cause gross alterations in the histological appearance very considerable damage must have been done In a highly

FIG 19 Tadpole skin. Syncytium formation after irradiation in presence of colloidal silver

delicate semi permeable membrane the probabilities are that changes in its physical functions would have occurred long before the manifestation of marked changes in its microscopical character

It is however unnecessary to labour the question of histological evidence since we have abundant chemical evidence of alteration in the permeability of the cell membrane in the case of red blood-corpuscles This membrane is known to have important properties it is normally impermeable to haemoglobin and to kations while allowing of the free passage of such anions as the chlorion and the bicarbonate ion Precisely what determines this specialization of function is not exactly known undoubtedly the Donnan equilibrium plays an important part the outstanding feature from our present view point is that radiation renders the membrane permeable to haemoglobin.

For β radiation this was shown as long ago as 1916 by

Hausmann, who found that blood-agar was haemolysed on exposure to the β radiation from radium. The experiments were from their character naturally of a somewhat rough kind but we have fortunately some more accurate observations upon the action of X rays made by Pickering and Collins (1923) Human red blood-corpuscles were employed and 5 per cent suspensions made in physiological saline solution Locke Ringer s solution and in isotonic gum saline for each experiment 10 c c of the suspension were used

A medium focus Coolidge tube running at 90–125 kv and 5 milliamperes with a constant distance of 22 cm from the surface of the suspension was the source of the X radiation and exposures were made for 10, 20 and 30 minutes Crenation and haemolysis were observed in the suspensions made in physiological saline and Locke-Ringer solutions, but haemolysis was only perceptible to the sight after long exposures On centrifuging the irradiated specimens and examining the supernatant fluid by the benzidine test the presence of haemoglobin in the supernatant liquid was nevertheless established In the isotonic gum saline medium crenation and haemolysis were absent and though in a few cases some of the corpuscles appeared to be spherical and swollen the majority seemed normal and rouleaux persisted even after a 30-minutes' exposure.

Koch (1927) made examinations of the chloride content of red blood-corpuscles in the human subject after irradiation. Twenty cases were reported upon and the determinations made immediately after exposure In all cases there was found evidence of water inflow and in the majority of cases there was also a chloride migration into the corpuscles

Goulston (1932) confirmed the haemolytic action of β radiation and showed that there was only slight haemolysis after exposure to the γ-rays from 120 mgm of radium element for 119 hours

The observed facts clearly point to a disturbance of the physical functions of the limiting membrane

The Cytoplasm and Cytoplasmic Inclusions

Attention has been so usually directed to the nuclear changes following irradiation that mention of changes in

the cytoplasm itself has perhaps been the exception rather than the rule Marked changes can, however sometimes be observed when irradiated tissue is carefully compared with controls These can be seen in freshly removed rat s liver exposed to β-radiation (p 47) Here the cytoplasm of cells in the vicinity of the needle track showed a peculiar ground glass like appearance which suggested the formation of an exceedingly fine precipitate in the fixed and stained specimen. The appearance was in marked contrast to that seen in the controls The staining was also more diffuse and the sharp differentiation between granules and cytoplasm was obscured and in some cases lost The granules in the liver cells lost their sharp outline and the appearances suggested an escape of their contents into the general cytoplasm giving rise to a diffuse staining with eosin In some cases the granules appeared to have visibly ruptured, and in others coalesced, while generally there was irregularity of contour

Changes of a similar though much less marked character were observed in some of the cells in the case of strongly irradiated chick embryos As regards cytoplasmic inclusions other than granules the mitochondria and Golgi apparatus must next be considered.

The Mitochondria Nürnberger (1923) observed a diminution in the number and a clumping together of the mitochondria in the irradiated ova of white mice

Wail and Frenkel (1925) reporting on the action of X rays upon the mitochondria in the hepatic cells of the frog stated that the earliest changes which they observed as the result of exposure were in the mitochondria Within four to eight hours after irradiation the mitochondria lost their normal rod like form and showed commencing fragmentation into granules In 24 hours granules were the predominant and after 2 3 and 4 days the only forms of mitochondria present. The granules tended to lose their original more or less orderly arrangement and to become swollen and vacuolated These observers especially state that commencing mitochondrial changes make their appearance before nuclear effects are demonstrated. The exact appearances varied

according to the dose of X rays administered and to the lapse of time after exposure

Ludford (1932) irradiated mouse tumours *in situ* using an applicator containing 58 mgm of radium element with a silver screen of 0 18 mm in thickness, the duration of the

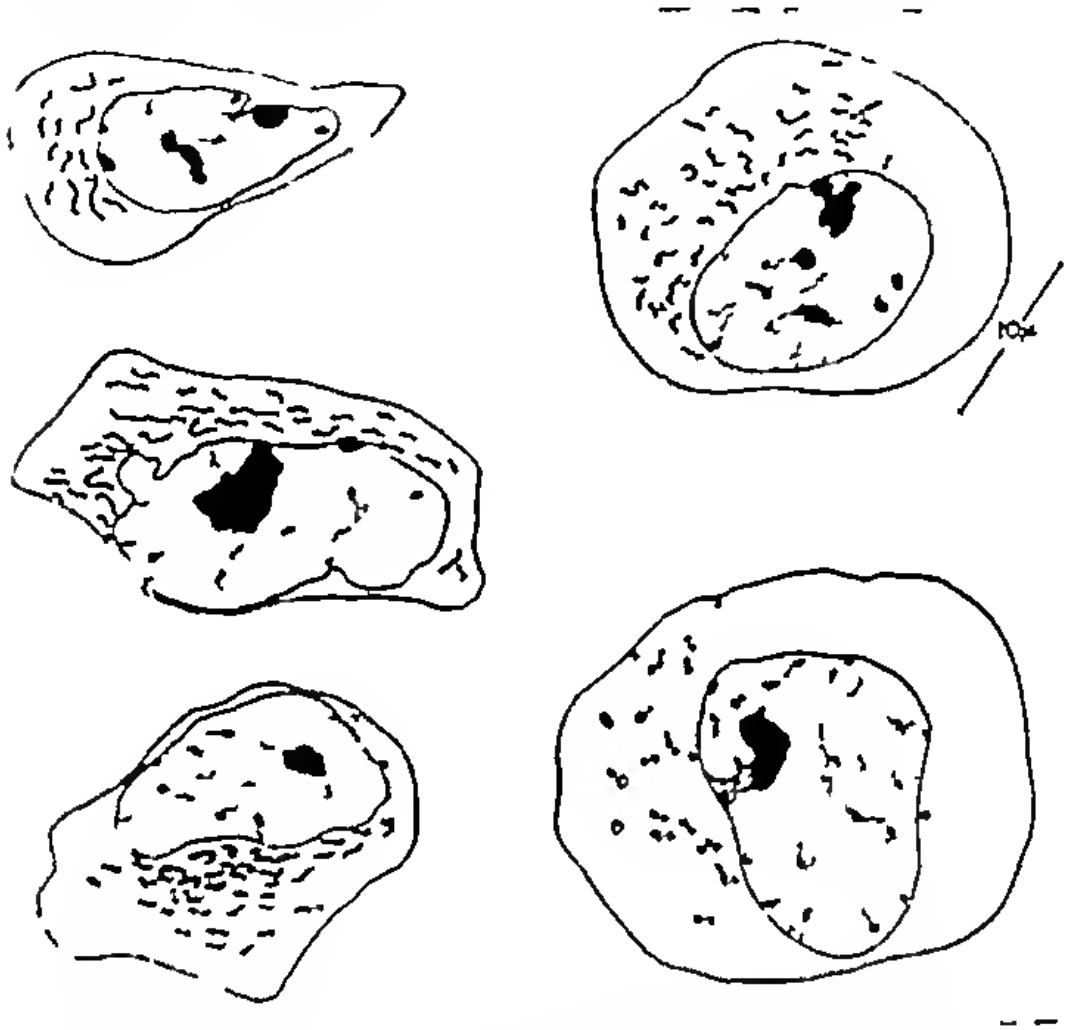


FIG 20 Changes in mitochondria after irradiation Gradual fragmentation and eventual vacuolation. (Ludford, *10th Scient Report Imp Cancer Res Fund* 1932)

exposure being one hour Obviously marked β ray effects were to be expected

The methods employed for mitochondrial staining were as follows

The modified Schridde method (Ludford 1931) staining with iron-alum haematoxylin after bleaching sections with hydrogen peroxide Also Champy fixation, followed by three days in saturated potassium bichromate solution and three

days in 2 per cent osmic acid Staining with iron alum haematoxylin and Altmann's aniline acid fuchsin.

Irradiation of the mammary adenocarcinoma number 27 caused the mitochondria in nearly every cell to become either granular or vesicular within 40 minutes This tumour was one which was not markedly radiosensitive and the dose administered was insufficient to cause its permanent regression The appearances presented by the mitochondria at different periods after radiation may be summarized as follows

Mouse adenocarcinoma 27 (mammary)

(i) *40 minutes* In some cells reduced in number and in practically all, cells either granular or vesicular
(ii) *4½ hours* In almost all cells granular or vesicular though some cells show filamentous forms
(iii) *7 hours* The cytoplasmic organs are not perceptibly different from those of the control preparations
(iv) *24 hours* Enlargement of cells has led to an increase in the number of mitochondria, which tend to be more filamentous than in the controls
(v) *50 hours* Mitochondria tend to be filamentous and more numerous in the larger cells.
(vi) *3 days* Very conspicuous and filamentous thus indicating a high degree of metabolic activity
(vii) *6 days* Degenerative changes more marked than at 3 days.

By the sixth day growth of the tumour had occurred since radiation it was estimated that it had doubled in size Mitochondria were generally filamentous but some vesicular forms were seen By the fourteenth day the tumour had increased to four to six times its size at the time of radiation and by the twenty second day to eight times that size Cytological examination at this stage showed no differences between the mitochondria and those of the controls

Sarcoma No 37

This was stated to be a radiosensitive strain of tumour

The mitochondria of actively growing cells are in the form of fine filaments and are spread throughout the cytoplasm but in the less active cell both *in vivo* and *in vitro* they

tend to be granular and to become collected around the sphere During all degeneration they swell up and finally become vesicular (Ludford)

(i) *6 hours* after irradiation. The mitochondria tend to become granular or swollen. They are sometimes reduced in number

(ii) *48 hours* The mitochondria differ in their form and arrangement. In the smaller cells they are filamentous and granular as in the controls. In the larger cells they are more numerous and tend to be longer while in the largest cells they have begun to collect around the central body and to revert to the granular form, a condition indicatory of quiescence

(iii) *72 hours* The mitochondria of enlarged cells are granular or vesicular and collected at one side of the nucleus. They sometimes appear to form finely granular bodies scattered around the Golgi apparatus.

(iv) *4 days* The mitochondria show the degenerative changes already described, or have completely disappeared.

(v) *5 days* At this period degenerative and necrotic cell changes were more advanced. Complete regression of this tumour usually follows the dose of radiation administered.

From these two sets of experiments it would appear that in less radiosensitive tissue the mitochondria though at first suffering degenerative changes afterwards assume a normal form. In the more radiosensitive tumour just described the changes are progressive and lead to disintegration and disappearance The mitochondria showed progressive degenerative changes

Some experimental work carried out at the Middlesex Hospital in 1910–11 with which Beckton and Russ were associated has been so frequently misquoted that a brief reference may be excusable At that time the study of mitochondria was but little advanced and it was thought that Altmann's granules —*which are probably mitochondria*— might be of diagnostic value in the histological examination of malignant or questionably malignant tissues The experiments in question were carried out on tissue after it had been removed from the body and are therefore not comparable with the experiments which have hitherto been described in this connexion. Thin slices of the tissue under examination —the spleen liver and kidney of the mouse—were exposed

to radon in some cases the α particles were allowed to act while in others they were screened off leaving only the β and γ-radiations It is not necessary to describe the experiments in detail here but the α-particles were potent in causing the disappearance of granules normally stained by Altmann's method while when the α particles were eliminated exposure to the β- and γ-radiations did not cause this effect

This work on tissue removed from the body has caused the writers mentioned to be incorrectly reported as saying that β-radiation was without effect on mitochondria the name of which is not even mentioned in their communications

Ludford's work definitely suggests that in the case of the less radiosensitive material the mitochondrial changes are reversible while in the more radiosensitive tissues which undergo complete regression as a result of the dose of radiation given, they are irreversible and their course is one of progressive degeneration

The Golgi Apparatus Studies upon the effects of radiation on the Golgi apparatus were made by Ludford (1932) using the same material, radium applicator and exposure as in the case of his work on mitochondria

In a mammary adenocarcinoma of the mouse (No 27) the cell arrangement is not usually acinar but in places well developed acini are present In this case the Golgi apparatus occupies a position between the nucleus and the lumen, while the filamentous mitochondria are arranged around the nucleus more or less vertically to the free surface of the cell. In the tumour under consideration the Golgi apparatus consists of irregularly shaped bodies either collected at one side of the nucleus or scattered through the cytoplasm In some instances they may anastomose and form a reticulate structure The individual elements which together form the Golgi apparatus have been termed Golgi bodies

In some cases the cells of this mammary adenocarcinoma produce secretion and when this is the case the commencing deposition of secretion is seen to be in close association with the Golgi apparatus when the amount of secretion is large the mass is surrounded by Golgi bodies In degenerative cells

the Golgi apparatus shows varying degrees of degeneration and fragmentation.

The main changes occurring in the Golgi apparatus of this tumour at different periods after irradiation, as given by Lud ford are as follows

(i) *40 minutes* The Golgi bodies are more rounded and less clearly defined than in the control
(ii) *24 hours* Many cells show masses of secretion associated with the Golgi bodies just as in non irradiated controls.
(iii) *50 hours* The Golgi bodies are still associated with the secretion, but are becoming more granular and fragmented, while they also show a tendency to break away from the mass of secretion.
(iv) *5 days* The Golgi bodies are enlarged more granular and less sharply defined than normal
(v) *6 days* The Golgi bodies are more rounded and granular
(vi) *14 days* The Golgi apparatus is still enlarged
(vii) *22 days* The appearances are normal

In the radiosensitive tumour Sarcoma No 37 the sequence of events is different as may be seen from the following summary

(i) *6 hours* Golgi apparatus shows no change
(ii) *48 hours* In some cells the Golgi apparatus is enlarged but in others it is apparently unaltered.
(iii) *79 hours* Although the Golgi apparatus may be enlarged, it is partly or completely broken up into granules.
(iv) *4 days* The Golgi apparatus shows marked degeneration in degenerate cells, being disintegrated into granules or rodlets.

Thus the influence of radiation upon the Golgi apparatus appears to be somewhat similar in character to that upon the mitochondria the tendency being to granulation and frag mentation The subsequent changes depend upon the radio sensitivity of the cell in radiosensitive cells the changes progress to complete disintegration while in less radiosensi tive cells recovery to the normal form may occur

The changes occurring in human carcinomata will be considered subsequently

The Nucleus

The action of radiations upon the nucleus will be most conveniently described under two headings

A The interkinetic or so-called resting nucleus

B The nucleus in mitosis

A. *The interkinetic nucleus* The effects of radiation have been studied in two distinct classes of material namely in tissues irradiated in the living animal (Ludford 1932) and in tissue freshly removed from the body (Colwell and Gladstone 1933)—tumour cells in mice (Ludford) and the cells of chick embryos irradiated *in ovo* (Gladstone and Colwell) The former of these may have the first consideration. Ludford used the same material and dosage as have been previously described (p 76) Dealing with the mouse adenocarcinoma No 27 he says that

> the nucleus of a typical cell contains a relatively large nucleolus or plasmosome This has a greater affinity for acid than for basic dyes, in contrast to the chromatin proper which stains more readily with basic dyes The former is usually referred to as oxychromatin, the latter as basichromatin.
>
> When the Feulgen reaction for the demonstration of thymus-nucleic acid is applied to the cells of this tumour granules of chromatin give an intensely purple colour the positive reaction of this test The linin network is stained lightly purple but so faintly that if sections are counterstained with light green a green colour is then acquired The plasmosome either gives a negative reaction, or is stained lightly purple but around its periphery are usually seen deeply stained granules, and sometimes a thin, darkly stained shell At the early prophase of mitosis the chromosomes are coloured intensely purple The plasmosome disappears from view the material of which it is composed being apparently incorporated with the chromosomes The latter retain their bright purple colour to the late telophase until the daughter nuclei are formed
>
> Around areas of necrosis in the tumour the degenerating cells have shrunken nuclei with irregular outlines Shrinkage results in what remains of the nuclear structure appearing intensely purple Commonly the chromatin granules run together to form droplets, and complete confluence of such droplets results in a uniformly purplish nuclear remnant Finally cellular disruption results in purple-stained fragments of irregular shape lying among the debris of dead cells.

The nuclear changes produced by radiation here described are mainly those in the interkinetic nucleus but references are also made to changes in the mitotic figures when these occur in the sections examined.

At different periods after exposure the findings are stated as follows

(i) *40 minutes* A very few abnormal mitoses are seen, with chromosomes clumped together as though cell division had been arrested before completion

FIG 21 Normal and irradiated nuclei (4 hours after irradiation) of adenocarcinoma No 27 (Ludford) Both stained by Feulgen method. (Ludford *10th Scient Report Imp Cancer Res Fund*, 193–)

(ii) *4½ hours* No mitotic figures seen

(iii) *7 hours* Some nuclei give the impression of being shrunken in comparison with the controls.

(iv) *24 hours* With rare exceptions, mitotic figures are absent. There

is an increase in the bulk of the nuclei without any corresponding increase in the chromatin granules. The meshes of the linin network appear distended. In sections counterstained with light green the appearances in some of the cells give the impression that there is a relative decrease in the purple staining constituents of the nucleus and an increase in the green-staining elements.

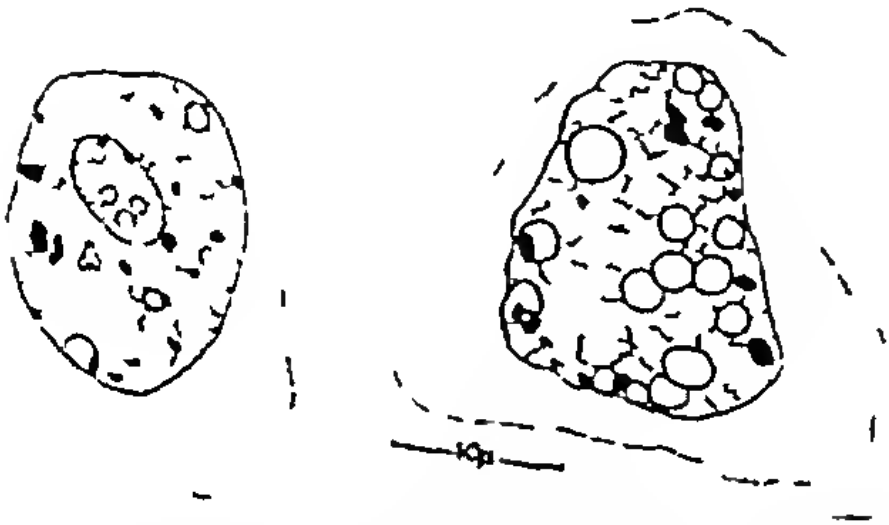


FIG 2.—Nuclear disintegration three days after irradiation (adenocarcinoma No. 4). (Ludford, *10th Scient Report Imp Cancer Res Fund* 193.)

(v) *30 hours* Some large nuclei present the appearances just described while others are hyperchromatic with a rather close reticulate structure staining a bright purple in the Feulgen preparations. More cells are now seen in mitosis some are definitely abnormal while others do not show any difference from the control In the latter case the chromosomes are normal in appearance and distribution, and give a normal reaction to Feulgen's test

(vi) *3 days* At this period the post irradiation changes generally are at a maximum. Many cells have attained their maximum size and degenerative changes are marked Variation in the appearances of cell are nevertheless strongly marked side by side with cells showing very pronounced degenerative changes are others of quite normal appearance Mitosis is observed in cells of all sizes but the mitotic figures are often abnormal Some cells are multinucleate The appearances suggest that in some cases abortive mitosis has resulted in incomplete separation of daughter cells. With Feulgen preparations degenerative nuclear changes are seen to consist

of vacuolation which in advanced cases may be so marked that the vacuoles form the predominant feature and the remains of the chromatin are collected into large granules. The appearances strongly suggest that chromatin has undergone transformation into an achromatic substance.

(vii) *5 days* The general appearances are much as described in the previous specimen but an impression is given that there is an increase of chromatin in some of the cells, followed by its disintegration into the achromatic substance of the vesicles. Abnormal mitoses occur; the chromosomes showing malformation, fusion, failure to become attached to the spindle and lagging during the telophase On the other hand, normal mitoses are also seen.

(viii) *14 days* Abnormal mitoses continue to be seen sometimes a large number of chromosomes is present, and marked degenerative changes occur

(ix) *22 days* At this period after irradiation, the appearances are normal As mentioned before, the tumour is non regressive under the dose of radiation administered.

In tissue cultures exposed to X rays [1] Strangeways could observe no changes in the interkinetic nuclei of cells irradiated for shorter periods, but when the exposures reached 45 minutes or over, marked changes occurred. The nuclei become somewhat enlarged and the nucleoli may show in addition to an increase of size irregularity of outline and such irregularities of staining as to produce a granular appearance At the same time the cytoplasm enlarges and becomes less dense and more granular With very lengthy exposures some cells show varied and complex outlines followed by fragmentation With lengthening exposures to X rays not only are the changes produced more marked, but greater numbers of cells are affected.

The experiments of Colwell and Gladstone (1933) may next be considered. In these the experimental material was the freshly removed liver of the rat taken under the same conditions as have been mentioned in the section on autolysis The liver tissue was thus fresh and at the same time its removal from the body and the low temperature at which it was kept during exposure to radiations render it probable that observed changes are rather of the nature of simple

[1] See p. 91 for details of exposure.

physico-chemical actions than of any response to the radiations by the living cell or due to the combined action of radiations and intracellular enzymes

As in the autolysis experiments (p 51) the freshly removed pieces of liver after being plunged into ice-cold saline and transfixed with the radon tube were placed in the cold chamber for varying periods They were then fixed in Bouin s

FIG 3 Low power view showing local effects of exposure to β-radiation. The large oval space shows the position of the radon tube (*Gladstone*)

solution and afterwards stained with eosin and haematoxylin in the usual manner

The most striking effect of exposure to radon when this is enclosed in thin walled glass tubes by which the tissue is transfixed is marked nuclear pycnosis in the vicinity of the active source associated with this are the changes in cell outlines cytoplasm and granules to which reference has already been made The low power appearances are shown in the accompanying figure there is of course a certain amount of compression in the immediate neighbourhood of the radon tube and this of necessity gives rise to a crowded effect of the nuclei There are however outstanding and

characteristic changes in the nuclei themselves which are shown in

1 Staining effects
2 Nuclear membrane
3 Nuclear structure

1 *Staining effects* With the ordinary haematoxylin stain these are characteristic and different from those seen in the earlier stages of autolysis With autolysis nuclear pycnosis occurs but the chromatic material then has a purple tint. With exposure to radon without any autolysis the nuclear pycnosis is associated with a peculiar blackish tint which has not been observed under any other conditions The appearances are quite distinct from those caused by pressure effects due to the insertion of a dummy needle in which case the nuclei take on the normal bluish purple stain sections of tissue exposed to radon can easily be distinguished by the colour of the nuclei when compared with the controls. In the immediate vicinity of the needle track practically all the cell nuclei show this blackening The zone of intense action extends approximately to about half the diameter of a liver lobule Outside this area is one in which the changes, though perfectly definite are by no means so marked many cells showing a tint intermediate between the black and normal appearances It is worthy of note that all nuclei do not show the same degree of change especially just beyond the zone of intense action Here while many nuclei show the definite blackening the majority are of an intermediate type while some appear perfectly normal. It will be remembered that throughout Ludford's series of experiments with mouse tumour a similar variability in the nuclear reactions was noted and the two sets of experiments lead to the conclusion that variations in susceptibility are present even in the absence of definite mitoses In the zone of most intense radiation however the changes are universal.

2 *Nuclear membrane* In the zone of most intense radiation shrinkage and blackening quite obliterate any other features but where radiation is less intense irregularity and thickening of the nuclear membrane are conspicuous

3 *Nuclear structure* When nuclear structure is visible the chromatin network is less regular while the strands are less regular in outline and more deeply stained than normal

The vacuolation seen in the later stages of the nuclei in Ludford's specimens was not observed in this series of experiments as will have been noted this was in all cases a comparatively late phenomenon. In order to form some idea of the length of exposure to radon necessary to produce changes in the cell nuclei of freshly removed liver a series of specimens was transfixed with radon tubes (10 mc approximately) these were placed in the cold chamber and specimens removed and fixed from time to time Definite changes were observed in specimens removed after two hours exposure and the changes increased in intensity with lapse of time The experiments certainly point to physico-chemical changes in the nuclear substance as the result of exposure to radiation (see p 49)

Gladstone and Colwell (1933) also observed pycnotic changes in the nuclei of the tissues of chick embryos given massive doses of X rays (4 P.D Sabouraud) The changes were not observed in all the nuclei but some appeared to react differently from others Thus in the same field and the same tissue certain nuclei would show varying degrees of pycnosis while others were apparently unaffected. Changes were also observed in dividing nuclei which will be considered subsequently

B *The nucleus in mitosis* The effects of radiation upon the chromosomes of nuclei in active mitosis were so obvious that they very early attracted attention. It must not be forgotten however that atypical mitoses were produced by various toxic chemical agents such as chloral hydrate quinine antipyrine ether and carbon dioxide long before the discovery of X rays or of radioactivity (Hertwig 1887) In view of their obvious relations to the therapeutic effects of radiation the nuclear changes due to this have necessarily been subjected to very minute scrutiny

Already in 1903–4 Perthes had noticed abnormal mitosis in the ova of *Ascaris megalocephala* as the result of radiation and extension of his observations to mammalian normal and

neoplastic tissue confirmed his original findings He concluded that nuclei in active mitosis are hypersensitive to radiation, which also causes a cessation of karyokinesis

In 1905 Bergonié and Tribondeau laid it down as a general principle that actively proliferating tissues are specially sensitive to radiations a view which was almost immediately confirmed by the work of Regaud and Blanc (1906) and of Krause and Ziegler (1906)

A considerable amount of discussion has centred around the question as to which stage of the mitotic cycle is the most sensitive to radiation the following Table shows the views advanced by a few writers together with the type of experimental material employed

Author	*Date*	*Most sensitive phase.*	*Material.*	*Type of radiation.*
Mottram	1913	Metaphase	Ascaris ova	Radium β and γ
Regaud	1923	Prophase Anaphase	Various tissues including neoplasms	X-rays and radium
Holthusen	1921	Metaphase	Ascaris ova	X rays
Alberti and Politzer	1923–6	Metaphase	Cornea of salamander larva	X rays
Strangeways and Hopwood	1926	Prophase	Tissue cultures Choroid and sclerotic of chick embryo	Radium and X rays
Vintemberger	1928	Telophase	Ova of *Rana fusca*	X rays
Love	1931	The sensitivity of a cell — a function of its displacement from maturity	Tissue cultures Choroid and sclerotic of chick embryo	X rays

There is a fairly general agreement that cells in process of division are more sensitive to radiation than are cells in the interkinetic state Upon the most sensitive phase of mitosis opinions are widely divided In this connexion it may not be amiss to point out that the material employed is very varied various types of animal form have been the subject of experiment Vertebrate and invertebrate hot-blooded and cold blooded animals have all been used as well as the para

sitic *Ascaris* ova Perhaps during the actual visible stages of mitosis the sensitivity of the different phases may vary in different types

The experiments upon tissue cultures however point to the stages immediately preceding the visible prophase as being the most vulnerable From general considerations it is an experimental demonstration of what one might most naturally expect The conditions within the cell are in a state of temporary instability while the cell protoplasm normally undergoes a diminution in viscosity This in itself points to alterations in the state of the component colloids and it is precisely when such a state of unstable equilibrium exists that the ionizing effect of the radiations would naturally be most marked

Considering first the microscopical appearances of the chromosomes when cells in a state of mitosis are irradiated clumping fragmentation and irregular division and distribution are the outstanding features These forms of irregularity are well shown in the accompanying figures from Mottram's article His general conclusions from his experiments are as follows

1 As the result of the action of β plus γ-rays from about seven milligrams of radium bromide there occurs a disturbance of normal growth
2 This disturbance of normal growth is more marked if the cells during irradiation are in active division.
3 The dividing ova of Ascaris are at least eight times as vulnerable as the non-dividing ova
4 The most vulnerable stage in the division is the metaphase

β and γ-irradiation is followed by profound nuclear changes affecting the chromatin and such changes though present are less marked if the cells have been irradiated in a non-dividing condition

Reference has already been made to the very similar results obtained by other workers

For the purpose of examining the action of radiations upon the cells of warm blooded vertebrates and to eliminate such variables as blood and lymph supply possible nerve

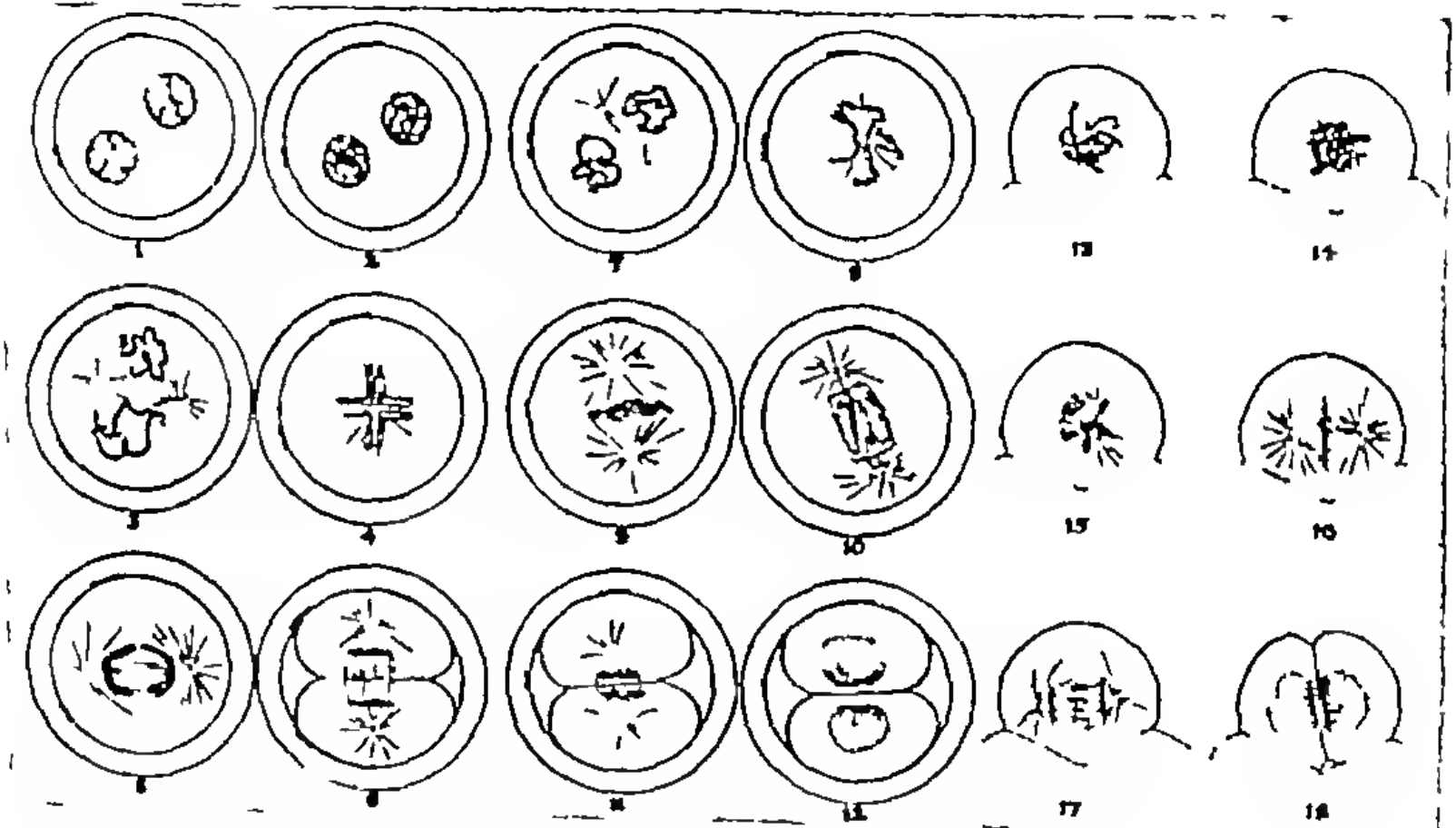


FIG 24 Mottram's experiments on mitosis in irradiated *Ascaris* ova. No. 1–6 controls: Nos. 7–12, nuclei after irradiation when in the interkinetic stage: Nos. 13–18, nuclei after irradiation during mitosis. (Mottram, *Middlesex Hospital Cancer Report*, 1913)

influences and the possible presence of antibodies Strangeways in 1920 began his studies on the action of radiations on tissue cultures

The earliest of these were carried out upon the action of X rays on tissue cultures derived from the choroid of the chick embryo and the articular cartilage of the adult fowl. For the purpose of irradiation the following experimental conditions have been thus given by Strangeways

The cultures are exposed at a temperature of 37° C to a beam of X-rays; a 1° inch coil is used with a spark-gap of 8 cm., and a gas tube with a current of 1 milliampere The cultures are placed at a distance of 24 cm. from the anticathode; the dose is regulated by varying the length of the exposure Under these conditions the incident beam is of such an intensity that 1 *e* (Friedrich) is given per minute Four cultures are exposed simultaneously and in this way changes noted in one can be controlled by comparison with the other three The cultures are returned to the incubator for 80 minutes before fixation and staining Any tissues may be used, but the present writer[1] usually employs the embryonic choroid and the adult articular cartilage of the fowl.

The changes observed vary as would naturally be expected with the duration of the exposure

Five minutes exposure Here the only observable change was a diminution in the number of prophase figures. Most cells already in prophase complete their division normally

Ten minutes exposure The number of cells showing late phases of mitosis is diminished. Definite changes appear in the chromosomes from metaphase onwards, and become more pronounced as the period of exposure is increased. Some chromosomes show variconty and fragmentation and granulation may follow When anaphase is reached the abnormal chromosomes divide unequally so that one daughter cell may receive more chromatin than another

Fifteen minutes exposure A lag in division is commonly seen in cultures which have been exposed for fifteen minutes or more. The metaphase may persist for one hour or more and in anaphase the chromosomes pass towards the spindle so slowly that the telophase is well advanced before the poles of the spindle are reached, so that anaphase and telophase are thus merged into a single stage The daughter chromosomes also show a lack of synchronism in their separation so that granules and fragments of chromatin may be seen lying on the spindle threads when cell division is nearly complete When the daughter nuclei are

[1] Le Strangeways.

completed these chromatin fragments are either left in the cytoplasm or extruded from the cell The end result is the production of apparently normal daughter cells, but in some cases one cell may be larger than the other

FIG 25 Cells from tissue cultures, showing normal cell division. (Strangeways, *Tissue Culture*, 1924)

Binucleated cells have been observed to arise from abnormalities in the telophase These abnormalities may be of three types

1 The daughter cells when almost distinct reunite to form a single binucleate cell.
2 The cytoplasm divides into two unequal portions one

of which contains both the nuclei and forms a binucleate cell The non nucleated fragment disintegrates

3 The processes of cell division are so altered as to disturb the normal separation of nucleoplasm and cytoplasm

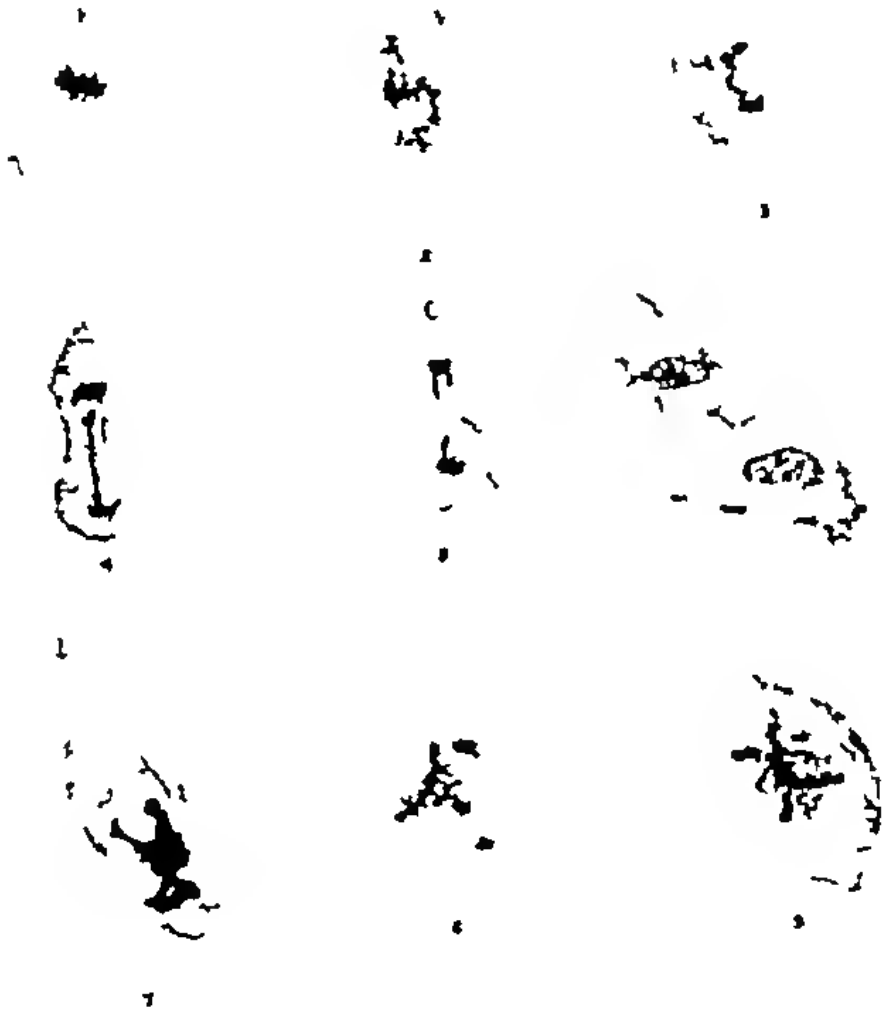


Fig 26 Cells from tissue cultures, showing alterations due to irradiation. (Strangeways, *Tissue Culture* 1924)

The nucleoplasm separates out into two or more portions and bi or even multinucleate cells are the result

Strangeways also employed a source of greater X ray intensity with a view to obtaining heavy dosage with brief exposure For this purpose he employed a standard Coolidge tube through which a current of 2 5 milliamperes is passed,

with an alternative spark-gap of 15 cm. and the cultures are placed at a distance of 15 cm from the anticathode In order to determine the existence or otherwise of a latent period before the changes already described manifest themselves four cultures were exposed simultaneously after which, two were fixed and stained at once while two were returned to the incubator for such period as might be necessary It was thus shown that there is a latent period of from 15 to 20 minutes before alterations due to irradiation become visible

A very important observation is the following

In all cultures in which the dose given is sufficient to effect changes, it is found that individual cells show marked differences in their reaction to the influence of X rays—indeed, no two cells can be said to respond in a precisely similar way This difference in reaction is found to be due in part to inherent physiological differences in the cells, and in part to the fact that the cells are in different stages of growth and mitosis at the time of exposure

This variability in sensitiveness or reaction to radiation, on the part of cells isolated from the influence of blood and lymph-supply or nerve control is very striking and sufficiently indicates the complexity of the problems attending the therapeutic radiation of neoplasms in the body How far the sensitiveness to radiation depends upon the exact stage of mitosis is as we have seen still a debatable point but premitotic and mitotic changes are themselves associated with physical changes in the cell and it seems in the nature of things probable that it is just at times of physical instability that the action of such agents as radiations will be most marked.

In the experiments of Colwell and Gladstone on the action of β-radiation upon liver cells removed from the body and kept at 0 C differences in nuclear staining were noted outside the zone of intense radiation immediately surrounding the radon tube Some of the cells showed marked pycnosis and the peculiar blackened appearance characteristic of nuclei exposed to intensive radiation while others appeared more or less normal. It does not seem likely that at 0 C—and the liver was plunged into a large volume of ice-cold saline im

mediately after removal from the body—active mitosis would be proceeding in mammalian cells and therefore as already said the changes observed seem to be due to a physical or physico-chemical sensitivity The existence of this of course depends upon the condition of the various cells at the time the tissue was removed from the body and affords corroboration of the remark of Strangeways that no two cells can be said to respond in a precisely similar manner

Subcultures after irradiation Subcultures from cultures which have received heavy doses of X radiation—300 *e* to 700 *e* (Friedrich)—show hypertrophy of non kinetic cells bipolar and multipolar mitoses and binucleated and multinucleated cells in some of which as many as twenty nuclei have been counted Cells undergoing multipolar mitosis may give rise to multinucleate cells

The experiments upon the action of radium radiations begun by Strangeways (1024) have been continued by his successors Irradiation was mainly carried out by means of two radium plaques containing 300 mgm and 100 mgm of radium element respectively Exposures were made in a specially designed radium lantern and stringent precautions taken to maintain a constant temperature throughout the experiments

With small doses of γ-radiation (100 mgm Ra element filtered by 0 5 mm platinum at a distance of 1·4 cm from the culture) there was found to be a fall in the number of cells starting mitosis soon after the beginning of the exposure This was followed by a return of mitosis after removal of the radium With increased dosage of radium abnormal mitotic figures began to appear similar to those described when the effects of X rays were under consideration

Alterations in the form of the chromosomes during mitosis and irregularity in distribution to the daughter cells render it hardly possible that these latter can be in all respects normal even though at the outset they may not show marked abnormalities So long ago as 1914 Boresl suggested the probability of such cells showing deficient viability and that if mitosis occurred subsequent generations would soon cease to live

Ludford (1930) confirmed the work of previous observers by showing that when tumour cells were irradiated *in vivo* the wave of mitosis following removal of the radioactive source was accompanied by marked abnormalities in the form and disposition of the chromosomes.

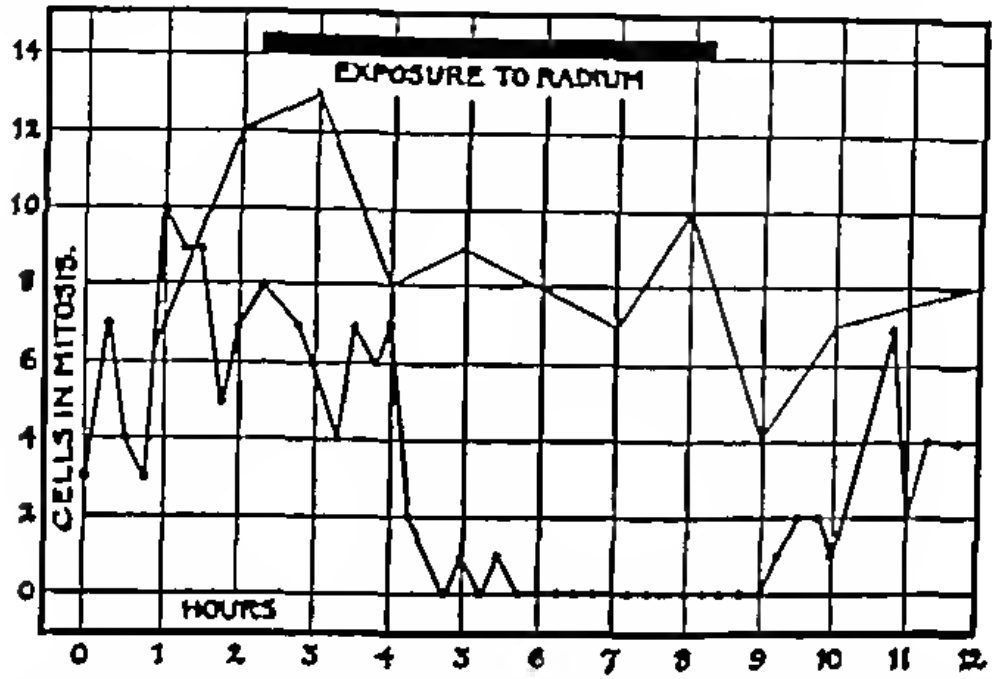


FIG 27 Graph showing cessation and return of mitosis in culture of chick choroid irradiated with 100 mgm. Ra. (0 5 mm. Pt. filtration) at 1 4 cm. distance. The thin line shows the number of cells in mitosis seen in a control culture of the same age. (Canti, *Acta radiologica*, 1929.)

In attempts to produce an immediate lethal effect by radiation, so that no cells in a tissue culture afterwards entered into mitosis it was shown by Strangeways and Hopwood (1926) that 17 000 *e* (Friedrich) units were necessary in the case of X rays As this corresponds approximately to 100 erythema doses it clearly falls outside the limits of tolerance exhibited by the human subject A similar result was recorded by Spear for the γ-rays of radium

It has accordingly been held by many that doses of radiation of the order used in therapy produce their action by a delayed effect in which case the dose of radiation affects mitosis so as to give rise to a line of cells of low vitality which die out in one or two generations

The fall in mitoses resulting from irradiation and the subsequent recovery are shown in the accompanying graph.

The mitoses occurring after exposure are either themselves abnormal or give rise to abnormalities in the subsequent generations

Canti and Spear (1927) using the diminution of the number of cells in mitosis as indicator concluded

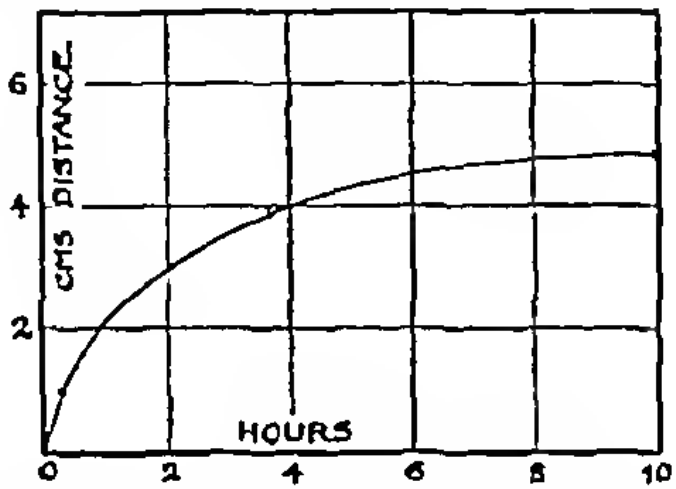


FIG 28. Graph showing the effect of varying the distance of the source of radiation in number of cells in mitosis. (Canti, *Acta radiologica* 1929)

1 That there is a threshold of intensity below which no effect is obtained however long the radium is left *in situ*
2 That there is a threshold of time for each intensity which must be passed before the effect is obtained

By varying the distance of the 100 mgm radium applicator (filter 0·5 mm platinum) the intensity of radiation could be varied in the accompanying curve the numbers of cells in mitoses are plotted against the time of exposure and distance The initial rise and subsequent flattening out of the curve are very distinctive

Spear (1930) working with tissue cultures found that if the dose were divided its effect was greatly enhanced much more effect was produced than if the same intensity of radiation had been administered in a single dose In Spear's work quite short exposures were given e g 2·5 minutes thus when 100 mgm of radium are used two irradiations each of 2·5 minutes duration have a much greater effect upon mitosis than a single exposure of five minutes The effect of the divided radiation was of the same order as a single

irradiation of 2 5 *minutes with 300* mgm of radium This interval of 80 minutes between the irradiations was found to be the optimum, an interval of 160 minutes being much less effectual With repeated exposures to 300 mgm Ra (0 5 mm Pt filter) at 0 5 cm distance it was found that 24 exposures, each of 2 5 minutes separated by 80-minute intervals (total exposure = 1 hour) produced a delayed lethal effect equivalent to that following a continuous irradiation of 4 5 hours

Eidinow and Mottram (1931) showed that Jensen's rat sarcoma is slightly more sensitive to divided than to continuous γ-radiation of the same total duration. Here however, the intervals between the exposures were measured in days

Spear (1931) showed that when tissue cultures are exposed to radium at 1-day intervals it is immaterial whether a lethal dose of radiation is given in one dose of 6 hours or in six equal fractions of 1 hour at 24 hourly intervals

Referring to experiments with tissue cultures Spear remarks

> The present work suggests, however that, at any rate with tissue cultures, effective use may be made of quite short exposures to radium, if the dose is frequently repeated at suitably short intervals, which can be measured in minutes. Since with such an arrangement of doses, cells are not prevented from entering *mitosis*, successive groups can be irradiated in the premitotic sensitive stage until the whole culture becomes degenerative.

Some work by Mottram (1930) upon non-disjunction of chromosomes in *Drosophila* is of special interest in connexion with what has already been said regarding the action of radiations in interfering with the ordinary oxidative and respiratory changes in cells He found that the occurrence of non-disjunction in *Drosophila* is greatly increased by subjecting nearly hatched females to the γ-rays from radium In a later communication made in the same year he found that the exposure of female flies before fertilization to carbon dioxide increases the occurrence of non-disjunction and probably of gynandromorphism Exposure to β-radiation from radium like exposure to γ-radiation greatly increases the occurrence of non-disjunction

Collagen Fibres

In addition to the effects of radiations upon cell structure must be mentioned their action upon collagen fibres A characteristic example of the swelling occurring in tissue of this type is seen in the accompanying figures which show the swelling produced by irradiation in the collagenous elements of a sarcoma of the abdominal wall in a hitch (Lacassagne and Monod, see p 101) Wolbach (1925) in his analysis of the histological changes occurring in radiation dermatitis regards alterations in the collagen elements of the subcutaneous tissue and blood vessels as very materially contributing to the lesions which occur and indeed states that collagen changes precede changes in the epidermis By this we suppose he means more or less obvious low power changes since nuclear changes can be observed directly after a radium applicator has been removed.

Wolbach began some observations on the alterations in the power of collagenous tissue to imbibe water as a result of radiation, and received the impression that this was increased so far as I am aware the experiments were not completed

From the work of Lacassagne and Monod (1922) Wolbach and others it is obvious that post radiation changes in the collagen elements whether of normal or of neoplastic tissue must have very marked effects upon the structures in which they occur

Thus far an account has been given of the main actions of radiations upon some of the chemical substances which are known to be most intimately connected with cell structure and it has been shown that all are more or less affected by exposure Similarly the various histological elements which make up the cell have also been seen to be more or less severely affected by radiation. Up to the present time comparatively little attention has I think, been given to alterations in the cell and nuclear membranes but it seems to me highly probable that these may be of fundamental importance Osmotic relations play the chief part in maintaining the system of electrolyte equilibria which is responsible for the maintenance of those buffer reactions so

essential to ordinary life processes particularly those of respiration and it must be borne in mind that obvious as are the histological changes seen in cell and nuclear outline as a sequel to irradiation changes in physical properties and functions may have taken place long before any such gross

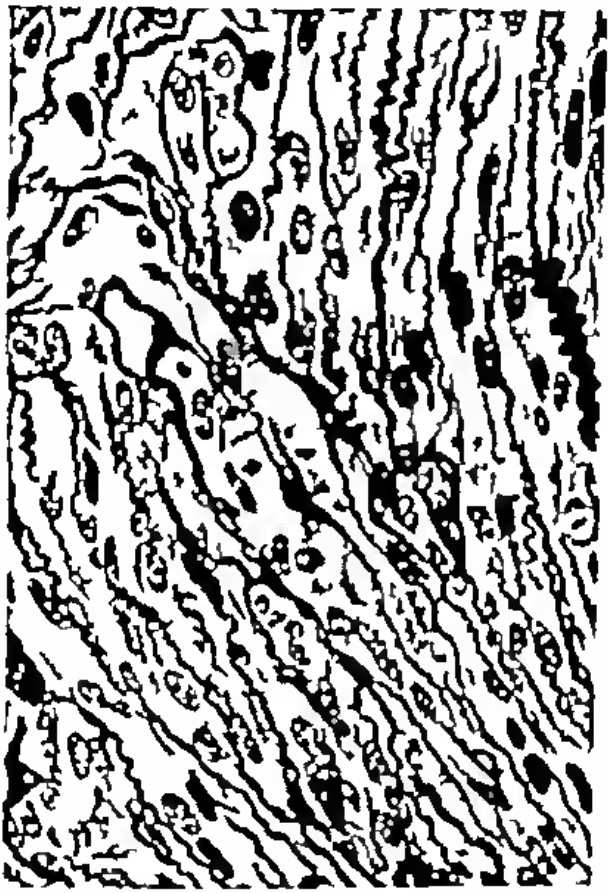


FIG 29 To show effects of radiation upon collagen fibres: Control. Compare fig 30 p. 101 (*Lacassagne and Monod, Archives de l'Institut du Radium.*)

anatomical lesions are manifest. Alterations of this character probably occur early and are of wide-reaching importance. As has been seen, they appear to be of such a nature as to increase permeability thereby influencing to a marked degree not only the electrolyte content of the cells but also autolytic processes by facilitating the removal of autolytic products It may be added that confirmation of the post-radiation changes in cell outline has been given by tissue culture experiments examined *in vivo* The changes observed in fixed and stained specimens are thus substantiated beyond doubt and the question of artefacts does not come in. Indeed,

the constant occurrence of changes in nuclear membrane and cell outline which are seen in irradiated material by whatever method it is examined, points conclusively to more or less profound physico-chemical alterations

FIG 30 To show effects of radiation upon collagen fibres Irradiated. (Lacassagne and Mosse, *Arch res de l'Institut du Rad ium*)

The constant occurrence of the blackish coloration seen in the nuclei of specimens exposed *in vitro* to intensive β-radiation and stained by haematoxylin is at least suggestive of primary chemical change whatever methods of fixation may have been adopted.

In the chemical section it was shown that not only do the proteins undergo changes when considered as physical systems but they also as a result of intramolecular change undergo a change in their reaction, becoming very definitely more acid in character This in itself would tend to increase autolysis in an injured cell and thus to facilitate its disintegration and removal.

As regards cell inclusions the mitochondria have been seen to be remarkably sensitive to the influence of radiations of their great importance to the life of the cell there can be no doubt whatever, whether they be connected as many hold with oxidation processes or with the general maintenance of cell functions Such cell inclusions as droplets and granules also show marked changes after radiation, of which irregularity of outline and coalescence to form larger droplets and granules are perhaps the most prominent. In cells which normally secrete mucin the effect of radiation is uncommonly striking and is well seen in the intestinal epithelium Twenty four hours after exposure to radiation such cells in the intestine of the rat can be seen to have undergone practically complete mucoid change with rupture towards the intestinal lumen Keratinizing changes also seem to be accelerated by exposure to radiations and indeed it does not seem too much to say that any process of protoplasmic degeneration which is more or less normally present is accentuated by irradiation.

Of glutathione oxidizing enzymes and oxidation reduction phenomena mention has already been made All are profoundly modified by exposure to β- γ- and X rays Such primary action as may be exerted by the rays themselves will undoubtedly be affected by alterations in electrolyte equilibrium due to changes in the cell membrane

In this connexion it may be suggested that the effect of radiations upon films both of simple substances and of such artificial complexes as may most closely resemble some of those occurring in the body would be a profitable and interesting subject of research

The reason why β-radiation has such an intense local destructive effect should be sufficiently obvious every chemical constituent of the cell is more or less altered and in the immediate vicinity of an unscreened radon seed there will be the local formation of hydrogen peroxide and possibly of other analogous substances Every structural unit of the cell is more or less injured so that cell death and tissue necrosis are all that can be expected With the γ- and X rays —especially with those of shorter wave-length—the changes

are more gradual and less indiscriminate we have here agents which are under the operator s control by careful adjustment of dosage both as regards time and intensity The main problem of radiotherapy is to adjust these factors so as to produce the desired result As regards the physical side of dosage it may fairly be said that our knowledge is good as regards the *biological effects* of these *physical doses* a vast deal has yet to be done.

CHAPTER IV

THE RETICULO ENDOTHELIAL SYSTEM AND SOME IMMUNITY REACTIONS

The reticulo-endothelial system is generally regarded as being specially concerned with the defence of the body against living and non living toxic agents, either by the process of antitoxin formation or by phagocytosis The term was first introduced by Aschoff in 1924 as a collective name for a system of cells distributed throughout the body and specially demonstrated by methods of *intra vitam* staining

When carmine is injected intravenously it is found that the dye is absorbed by

(*a*) Special cells of the spleen pulp
(*b*) Certain branched cells of the bone-marrow
(*c*) The Küpffer cells of the liver
(*d*) The reticulum cells of the lymph glands
(*e*) The interstitial cells of the testis

The typical endothelia of the blood vessels remain unstained, and partly at any rate on this ground the name has been adversely criticized by Maximow He adhered to the selective retention of injected colloids as a criterion of the cells composing the system as a whole and according to him the chief elements were located in the following situations

1 Resting wandering cells[1] (clasmatocytes adventitial cells) of the loose or dense regularly or irregularly arranged connective tissue and the serous membranes
2 The reticular cells of the lymphoid and myeloid tissue and the red pulp of the spleen.
3 The squamous cells lining the lymph sinuses in the liver marrow and spleen. The Küpffer cells in the liver and some cells in the walls of the venous capillaries in the adrenals and hypophysis cerebri.

It thus specially differs from other organs of the body in that the component elements are not aggregated into a single

[1] Resting wandering cells. This paradoxical-looking term is that used in several places by Maximow in his article in Cowdry's *Special Cytology* The term quiescent wandering cells is also used.

organ, but distributed more or less throughout the body The view that certain cellular elements of the loose connective tissue form part of *a widely distributed system* was first put forward by Goldmann

The cells—or certain of them—would appear to be capable under different circumstances of remaining fixed or of amœboid movement The terms histiocytes[1] and macrophages[2] have been used to describe them especially when in the amœboid state The destruction of effete blood corpuscles *is believed to be one of the functions* of this widely spread system which is responsible for the formation of bilirubin the iron free pigment isomeric with haematoporphyrin Effete cells and bacteria are submitted to the process of phagocytosis Landau and McNee (1914) consider the reticuloendothelial metabolic apparatus as intimately connected with the formation of cholesterol. It should be mentioned that in different situations the macrophages or histiocytes show more or less typical structural differences and that their microscopical appearance is greatly influenced by their functional condition and whether they are fixed in position or amœboid and wandering

The effect of the action of radiations upon this widespread system with its varied functions especially those of protection is obviously a matter of great practical interest

The Effects of Radiation on Antibody Formation

Almost as soon as the X rays were discovered it was suggested that in virtue of their penetrating power they *might be used therapeutically* to influence deeply seated pathological processes more especially pulmonary tuberculosis Tubercle bacilli themselves were exposed for long periods without any apparent effect and the general consensus of opinion was that as a therapeutic agent in the treatment of pulmonary tuberculosis X rays were of no value In *guinea pigs inoculated with tubercle bacilli* and subsequently treated with X rays the findings were discordant

[1] Histiocyte simply means tissue-cells (Kyrle 1914).

[2] Macrophages to distinguish them from microphages, or polymorphonuclear leucocytes.

some observers found that the normal fatal issue was retarded, others that it was accelerated In the case of tuberculous lymphatic glands—e.g those of the neck—chemical evidence showed that in a large number of cases beneficial results were obtained and it was early recognized that these were almost certainly not due to any lethal action upon the bacilli themselves A large number of cases showing localized pyogenic infections were treated often with markedly beneficial results. The lack of any accurate system of dosage was an obvious disadvantage when attempts were made to establish any systematic method of treatment Localized cutaneous pyogenic infections such as furunculosis and such conditions as paronychia were the most favourable cases for treatment it was, however noted that they gave a better response when they were treated at an early stage For some twenty years this more or less haphazard empirical therapy was practised with varying degrees of success but in 1915 Heidenhain and Fried were led to undertake a systematic research upon the subject The investigation which lasted for ten years was suggested by two accidental observations Two cases of post operative suppuration of some months duration were subjected to X ray examination by screening and skiagram To the surprise of those concerned the suppuration mysteriously disappeared after the X ray exposures The results of these investigations were published in 1924 and were found to concur with those of Holzknecht and Pordes who had been investigating the question independently It was found that

1 In about one third of the cases the inflammatory process was markedly shortened sometimes even to a mere fraction of its normal duration
2 In about one-third the disease ceased rapidly in about twenty four to forty-eight hours practically by crisis
3 In the remaining third there was either slight improvement or complete failure

The essentials of successful treatment as outlined by Holzknecht are as follows

1 *Dosage.* It is essential that the dose given should be small. As estimated on Holzknecht s dosimeter scale dosage falls into four groups

(*a*) Highest doses 8–15 H (sometimes exceeded for skin carcinomata where the normal skin tolerance is not considered)
(*b*) Medium doses 5–7 H.
(*c*) Small doses 2–4 H
(*d*) Minimum doses 1–2 H

It was the last or minimum dose administered through 0·5 mm of zinc brass or copper that gave the best results in coccus infections

2 *Time of dose.* The best results were obtained when the condition was treated in its early stage.

Serological examination showed that there was an increase in the bacteriolysin content of the blood of patients after irradiation. Pordes considered that the effects were due to a destructive action of the small doses of radiation upon the highly sensitive leucocytes whereby antitoxins and bacteriolysins were liberated.

Before the publication of these results a good deal of work upon the action of X rays on antibody formation and allied problems had already been carried out Benjamin and Sluka (1908) found that in rabbits exposure to X rays before the injection of ox-serum diminished or abrogated the formation of specific precipitins and at the same time they found that there was a delay in the disappearance of the antigen from the blood of the irradiated animal. If the radiation was given four days or so after the injection of the antigen (ox-serum) it was without any obvious effect upon precipitin formation. The experiment obviously points to the conclusions that

(*a*) If radiation is carried out before the injection of antigen, the mechanism whereby the antibodies are formed is either inhibited or completely thrown out of action.
(*b*) Within the doses given, radiation is without effect on the already formed antibody
(*c*) The means by which the antigen is removed from the blood is impeded in its action. It is now considered that it is the reticulo-endothelial system by which the disappearance of antigen from the circulation is effected.

Löwen (1909) found that the formation of specific bacterial agglutinin and bacteriolysin was restrained in irradiated

animals but that no action was produced on the agglutinin in the blood of normal animals or on typhoid agglutinin exposed to X rays *in vitro*

The effects of X radiation upon the occurrence of anaphylaxis in guinea pigs was studied by von Heinrich and his conclusions were given as follows

1 Animals to which a single erythema dose had been given soon after the preliminary injection reacted much less severely on subsequent injection than did control animals

2 Passive anaphylaxis could not be produced by injecting the serum of irradiated guinea pigs soon after the injection of the antigen. From this it was concluded that the action of X rays as seen in (1) is to interfere with the production of the body upon which anaphylaxis depends

3 In animals which received the second injection of antigen some six weeks after the first injection (which was immediately followed by X radiation) the symptoms were exceptionally severe It was suggested by von Heinrich that the phenomenon might be due to the retention in the body of the antigen in an available form so that when regeneration of blood and lymph tissues set in after the irradiation specific antibody was produced. It was pointed out by Hektoen that this was in accordance with the observation of Benjamin and Sluka of the persistence of the antigen in the circulation of irradiated animals

The earlier work upon the relations of immune-body formation and X rays was largely based upon the view that leucocyte destruction caused the liberation of antibodies Löwen was unable to obtain any definite evidence of the liberation of endoleucocytic enzymes after the destruction of leucocytes by X rays but he did find evidence that the resistance of the experimental animals was diminished

Murphy and Ellis (1914) found that both normal and splenectomized mice upon exposure to X rays were rendered more susceptible to bovine tuberculosis than non irradiated animals

Hektoen carried out an extended research upon these subjects the first publication of which was made in 1915 As experimental animals he used in the first instance white rats weighing from 60 to 80 grams The antigen used was sheep s blood and the specific antibody investigated was the haemolysin for sheep s red blood-corpuscles [1] In this series of experiments a current of 4 milliamperes was used in the secondary the spark-gap was 5 inches and the distance from the tube to the rats was 18 inches The duration of exposure was 8 minutes

Hektoen s main conclusions may be summarized as follows

1 Repeated exposures to X rays after injection of the antigen do not markedly interfere with the production of lysin when the exposures though sufficient to lower the leucocyte count have no definite ill effect upon the health of the animals

2 When the X ray exposures are begun some days before the injection and continued so that the leucocyte count is markedly diminished for 15 to 20 days after the injection of antigen the formation of lysin is markedly reduced This may occur without much obvious effect on the general health of the animal In these animals the spleen lymphatic tissue and thymus were markedly reduced in size and the bone-marrow was affected In the blood the proportion of granular leucocytes was raised but there was a general leucopenia

3 A single prolonged—and eventually fatal—exposure to X rays given immediately before the injection of antigen may prevent any lysin formation for about 8 days or the lysin may be produced after a protracted latent period of about 20 days

4 The results harmonize with the view that antibodies are produced in the spleen lymph glands and bone-marrow as these suffer most from exposure to X rays

Three years later (1918) Hektoen contributed a further paper upon the effects of X radiation upon antibody formation when it is in full progress i e a few days after the

[1] Intraperitoneal injections of a 10 per cent. suspension of sheep s red blood-corpuscles: Dosage 5 c per kilogram of body weight.

injection of the antigen As it was necessary to employ animals of such a size that they would stand repeated bleedings dogs of a few weeks old and rabbits weighing about 1 kilogram were chosen A Coolidge tube working at 5–6 milliamperes with an 8-inch spark-gap and a focal distance of 8 inches from the irradiated animals was used as the source of radiation.

Exposure of these rabbits and dogs to X rays at about the same time as the antigen[1] was injected showed that it may inhibit the production or reduce the amount of antibody just as in the experiments upon rats to which reference has just been made If, on the other hand X radiation is deferred until antibody formation is well established it appears to have little or no effect upon the amount of antibody in the blood.

In addition to this Hektoen observed that dogs irradiated in the full tide of antibody production seemed to have their *resistance to the action of the rays very decidedly increased*[2] Exposures of 15 to 20 minutes were given with but little obvious effect Hektoen remarked nevertheless that a great deal more work would be needed before any definite conclusions could be drawn.

In 1920 appeared a further paper by Hektoen and Corper in which experiments upon the influence of injections of thorium X on antibody formation was described Biological research upon the effects of thorium X dates from about 1912, and in the following year Lippmann and Plesch showed that the complement was unaltered in the blood of animals injected with thorium X, even though the dose had been sufficient to destroy practically all the circulating leucocytes.

Frankel and Gumpertz (1914) found that thorium X had very little effect on the production of typhoid agglutinin except when very large doses were given.

Corper (1919) found that one half the lethal dose of thorium X injected either seven days before or coincidently with the

[1] The antigen used in the case of the dogs was rats or goats blood; in the case of rabbits, sheep s blood.

[2] It was observed by Lawson (1916) that the skin tolerance was raised in cases of dermal infection by cocci (*Radiology* vi (1926), 163)

primary or secondary injections of antigen had no effect upon the anaphylactic reaction in guinea pigs He had previously (1918) shown that injections of thorium X produced no alteration in the normal course of experimental tuberculosis in the same animals

Hektoen and Corper (1920) in the study of the effects of thorium X on antibody production, used rabbits as the experimental animals sheep s blood as the antigen and the resulting precipitin and haemolysin as the antibody for investigation. It was found that in rabbits treated with thorium X in the early stages of antibody formation the precipitin might be reduced in amount even though no definite changes in the leucocytes could be seen. If the injection was made when antibody formation was well established no effect upon precipitin formation was noticed The effect upon the production of haemolysin was uncertain and herein the action of thorium X differs from that of benzene[1] and X rays which cause definite inhibition of haemolysin as well as of precipitin.

Corper and Chovey (1921) found that mice injected with thorium X showed a great increase in their susceptibility to pneumococci and haemolytic streptococci as was shown by the greater severity of the symptoms their fatality and earlier appearance together with the fact that the injected micro-organisms persisted longer in the blood of the thorium X injected animals

It may be mentioned in this connexion that the course of experimental tuberculosis in guinea pigs appears to be un influenced by the administration of leucotoxic agents (Corper 1918 Kellert 1915 1916 1918)

Hektoen and Corper (1922) on injecting the active deposit from radon (of the order of 8–10 mc per 1000 gram body weight) into rabbits and using sheep s blood as the antigen, found a diminished production of haemolysin but a less marked change in that of precipitin.

[1] It had been shown by Selling (*Ziegler s Beitrage*, 51 (1911), 567) that injection of benzene caused marked destructive changes in the lympho-poietic and haemopoietic organs. A good deal of work was done upon the subject just about this time, including the effects on antibody formation

Simonds and Jones (1915) about the time of the publication of Hektoen's earliest work on this subject gave the results of their experiments upon X rays and the production of typhoid agglutinin. Rabbits were given exposures of 10–15 minutes daily for three weeks when a single large injection of killed typhoid bacilli was given. They themselves mention the absolute lack of any attempt at dosage A soft tube of high penetrating power with the current always of the same milliamperage was used, but otherwise there was no other measurement of the activity of the rays They record as their experimental findings

1 The resistance of the animal was lowered within 6 hours
2 The formation of agglutinin was markedly lessened.
3 The effect upon bacteriolysin formation was uncertain but it was probably diminished
4 Complement and opsonic power of the serum showed no appreciable change

It will be noticed that all the preceding work was carried out before the recognition of the reticulo-endothelial system (1924) as a specific entity with very definite functions in the production of immunity nevertheless the results obtained would be in full accord with its damage by radiations. In view of more recent work the retention of the antigen in the blood mentioned by Benjamin and Sluka and the persistence in the blood of the micro-organisms recorded by Corper and Chovey in mice injected with thorium X are perhaps specially interesting in this respect

In some of the recent investigations upon the effect of radiations on this system advantage is taken of the fact that normally it is enabled to segregate various colloids and dyestuffs injected intravenously and thus to cause their disappearance from the circulation. When its functions are impaired this function is lessened or lost with the result that the injected foreign material remains in the blood in greater quantity and persists there for a longer time than is normal.

One such method—that of Adler and Reimann (1925)—consists in the intravenous injection of a solution of Congo-red and the subsequent examination by colorimetric methods of blood samples withdrawn at different times after injection

Jaffé and Berman (1928) criticized the use of Congo red as the injection material and indicator on the ground of its rapid elimination by the bile The matter was reinvestigated by Pohle and Davy who though considering trypan blue a more suitable agent were in favour of not entirely discarding the Congo-red method. They point out however that it must be used in sufficient amount Thus using 10 o o of a 1 per cent solution, no dye was found remaining in the blood soon after injection. If the dose was increased to 100 o o of the same solution the injections being spread over three days it was possible to show its presence in the blood For investi gating the functional integrity of the reticulo-endothelial system after X radiation, Pohle and Davy injected 10 o o of a 1 per cent solution of Congo red in normal saline solu tion into the ear vein of rabbits The artery was found suitable as the source whence the samples of blood were withdrawn at various intervals of 4 minutes 1 hour 2 hours up to 24 hours 2 o.o of blood were withdrawn at each time clotting and separation of serum were allowed to occur spontaneously after which the separation was completed by the centrifuge at constant speed. For estimation 1 o o of the serum so obtained was diluted with 1-5 o o of physio-logical saline solution and the tint compared with that of a series of controls in a Bausch and Lomb Duboscq colorimeter

These workers were particularly struck by the extra-ordinary variations in non irradiated rabbits which made comparison exceedingly difficult They however give a sum mary of their work as well as details of individual methods frankly stating that their results show discrepancies with those of earlier workers Broadly so far as they commit themselves to conclusions on the subject the findings are as follows

1 Dose 150 R over spleen and injection 1 hour later = more rapid elimination of dye
2 Dose 300 R over spleen followed by immediate injec tion = but little change from non irradiated animals
3 Dose 150 R to whole body and immediate injection = approximately normal
4 Dose 300 R to whole body and immediate injection = approximately normal.

5 Dose 150 R to whole body and injection 1 hour later = slightly increased elimination

A more rapid elimination, of course means a higher efficiency of the reticulo-endothelial system so that these experiments suggest that under the conditions of radiation used, exposure to X rays raised that efficiency At the conclusion of their remarks they comment again on the discordant results obtained, on the individual variations and on the necessity for using a large number of animals if anything like definite results are expected

Schwienhorst (1928) experimenting on rats by exposure to X rays and injecting known amounts of staphylococci into the femoral vein, found that generally speaking the action of the rays upon the reticulo-endothelial system is gradually to diminish its phagocytic power until it is finally rendered totally insufficient The animals were divided into five groups

(*a*) Injected and irradiated on the same day
(*b*) Injected on day following irradiation.
(*c*) Injected 2 days after irradiation
(*d*) Injected 3 days after irradiation.
(*e*) Injected before irradiation.

In rats injected irradiated and killed a short time afterwards (3 hours) phagocytosis generally was good in some it was increased and there was a more rapid disappearance of the organisms from the blood stream Animals injected three days after irradiation showed a lack of phagocytosis either by leucocytes or reticulo-endothelial cells together with a diminution in agglutinin formation. The interval of time elapsing between injection and irradiation was found to be of great importance in determining the degree of protective reaction. Schwienhorst suggests that the increase in the bacteriolytic power of the serum described by Heidenhain and Fried may be due to local injury of the reticulo-endothelial system no evidence of direct phagocyte stimulation was observed, and it was pointed out that this is in agreement with clinical observation as to the futility of irradiating cases of septicaemia and general infection, since in these cases the reticulo-endothelial system has reached a stage of exhaustion.

Benassi (1929) working upon the absorption of trypan blue by the reticulo-endothelial system in irradiated rabbits concluded that a stimulating effect on absorption was caused by moderate doses of X rays but that too heavy doses caused diminished activity or even complete destruction of the absorptive function.

Mischtschenko (1929) found that in rabbits the administration of an 100 per cent erythema dose one hour before injection hindered the elimination of trypan blue when injected into the ear vein.

All the writers upon this subject appear to concur in the view that in order to secure beneficial effects from radiation in cases of microbial inflammation it is essential that the dose administered should be a small one In localized infection a general consideration of the evidence rather points to a response on the part of the reticulo-endothelial system than to leucocyte destruction with consequent liberation of antibodies. This is further corroborated by the fact that chronic infections and more acute infections irradiated in their late stages fail to give the best response If leucocyte destruction were the most important determining factor a rapid clearing up of the condition might be expected in the chronic cases the lack of such reaction perhaps rather indicates local exhaustion of the reticulo-endothelial apparatus Moreover the experiments of Regaud and others tend to negative the view that the lymphocytes themselves are hypersensitive to radiation, and explain their disappearance from the circulation after radiation by action upon the lymphopoietic tissues A further point is the necessity for small doses of radiation in the treatment of microbial infections and the observation—which seems established—that heavy dosage inhibits or destroys the protective effect of the reticulo-endothelial system

The experimental work of Moglinitzky and Podljaschuk (1930) upon the so-called haemato-encephalic barrier (*sog hämalo-enkephalische Barriere*) may appropriately have a brief note here The haemato-encephalic barrier constitutes a protection for the central nervous system against toxic agents circulating in the blood and this is regarded as one

of the functions of the mesenchyme apparatus the particular tissue concerned has not yet been determined. The experiments now considered were performed upon rabbits and dogs, with varying doses of X rays (150 kv 3 mm Al.) and solutions of trypan blue (1 2 per cent) or of saccharated oxide of iron (2 per cent) were injected intravenously Under normal conditions neither of these substances would pass from the blood into the brain tissues proper but as soon as the brain is treated with repeated doses of X rays both trypan blue and saccharated ferrous oxide may appear in certain parts of the brain. The brains of young animals were very much more sensitive than those of adults as was shown by degenerative atrophic changes in the mesenchyme apparatus in the former while they were not visible in the latter With divided dosage both trypan blue and ferric oxide were absorbed in the endothelium and perivascular glial elements this was regarded as due to a change in the permeability of the vascular system

CHAPTER V

RADIATION AND RESISTANCE TO TUMOUR GROWTH

On the subject of immunity to transplantable tumours an enormous amount of very controversial literature has accumulated. Woglom made two comprehensive reviews the one in 1913 and the other in 1928. For the latter he analysed the contents of some six hundred papers upon the subject and came to the conclusion that only a very small proportion were of any lasting value. Russ in 1922 gave a review to date of the work bearing upon the relation of radiation to immunity phenomena. A very brief note upon the latter subject is all that can be attempted here.

A short account of the effects of radiation upon the familiar subject of resistance to micro-organisms heterologous proteins and toxins has already been given, and the relations of the reticulo-endothelial system to these phenomena discussed.

The experimental observations that resistance to the growth of transplantable tumours could be increased either by a previous tumour growth or by the injection of such material as embryonic skin naturally raised the question of the production of cancer immunity and of its possible clinical applications.

Hitherto it has not been found possible to procure an immunity to tumours by the injection of an anti-tumour serum prepared on the same lines as anti-sera for diseases of microbial origin, or for agglutinin and the like. Moreover attempts to prepare specific precipitins for neoplastic tissues have likewise failed. In these cases there is an obvious difference from tumour immunity since the antigen is of foreign origin while in spontaneous tumours at least the neoplasm is derived from the tissues of the tumour bearing individual. There would seem to be a general agreement as to the protective mechanism in both sets of cases namely that in some form or other it depends upon the reticulo-endothelial system.

The importance of close attention to details of technique has been specially emphasized by Chambers and Scott, as well as by other writers To a lack of appreciation of the importance of often seemingly minor details a large proportion of the discrepant findings are probably to be attributed.

It was first pointed out by Russell (1912) that not all transplantable tumours are capable of producing immunity further there are marked differences between the life histories of tumours Some grow rapidly, metastasize freely and are rapidly fatal. Others are of slow growth and tend but little to the production of metastases Obviously discrimination is necessary when problems of immunity are under consideration

Although it was soon well recognized that inoculation of an animal with tumour material might lead to immunity it was none the less obvious that in many cases such engrafted material died more or less rapidly and underwent autolysis. The question then arose as to whether the immunity effects were due to the young actively growing neoplastic cells, or to the disintegration products of cells undergoing autolysis. Haaland (1910) demonstrated that tumour cells disintegrated prior to inoculation by freezing and grinding did not produce immunity and the same has been repeatedly found when dead or necrosed tumour tissue is injected there is here an obvious and marked difference from the production of agglutinin by the injection of dead cultures of micro-organisms

Chambers (1922) showed that if *healthy* cells from a Jensen's rat sarcoma are inoculated into a series of normal rats only a slight degree of concomitant immunity is produced. On the other hand if the injected cells have previously been damaged as for instance, by irradiation the degree of immunity conferred may be a high one

Evidence gradually accumulated has led to three conclusions

1 That young actively growing tumour cells do not produce immunity
2. That injured or degenerating cells may produce immunity
3 That dead or disintegrated cells do not produce immunity

Since radiations are known to cause the disappearance of neoplasms both spontaneous and inoculated the question of the possible production of an immunity producing phase in the course of the disappearance of the neoplasm is obviously one of more than theoretical interest So long ago as 1910 it had been shown by Contamin that a considerable amount of immunity was conferred upon mice by inoculating them with cells of tumour B which had been exposed to X rays and he further added the important and significant fact that a too-prolonged exposure to the rays destroyed the immunizing power of the irradiated cells

Wedd and Russ (1912) showed that when the cells of Twort mouse carcinoma are irradiated *in vitro* by the β- and γ-rays from radium bromide with a source of the intensity of 2 2 mgm per square centimetre for periods of an hour and upwards the tumour material does not grow when inoculated into normal but susceptible mice

Wedd, Morson, and Russ (1914) exposed thin slices of tumour tissue to β- and γ-rays in the same manner as before and inoculated fragments (0·1 c c) into a number of normal mice After fifteen days test inoculations with fragments of similar but non irradiated tumour were carried out and at the same time control mice were inoculated The numbers of tumours developing in the two sets of animals were then compared. The results are shown in the accompanying table

Duration of exposure to rad. em.	*Percentage of takes*		*Extent of immunity produced.*
	Irradiated	*Controls*	
	per cent	*per cent*	
1 hour	5 9	74	9*
2 hours	15·4	83	82
4 hours	20·0	53	63
6 hours	33 0	61	46
12 hours	54 0	78	31
24 hours	50 0	50	0

These results are strictly comparable to those obtained by Contamin as the result of exposure to X rays and the general conclusion reached is that mice may be made immune to inoculation of Twort carcinoma by the injection of previously

irradiated tumour cells but that too prolonged irradiation destroys this immunizing power

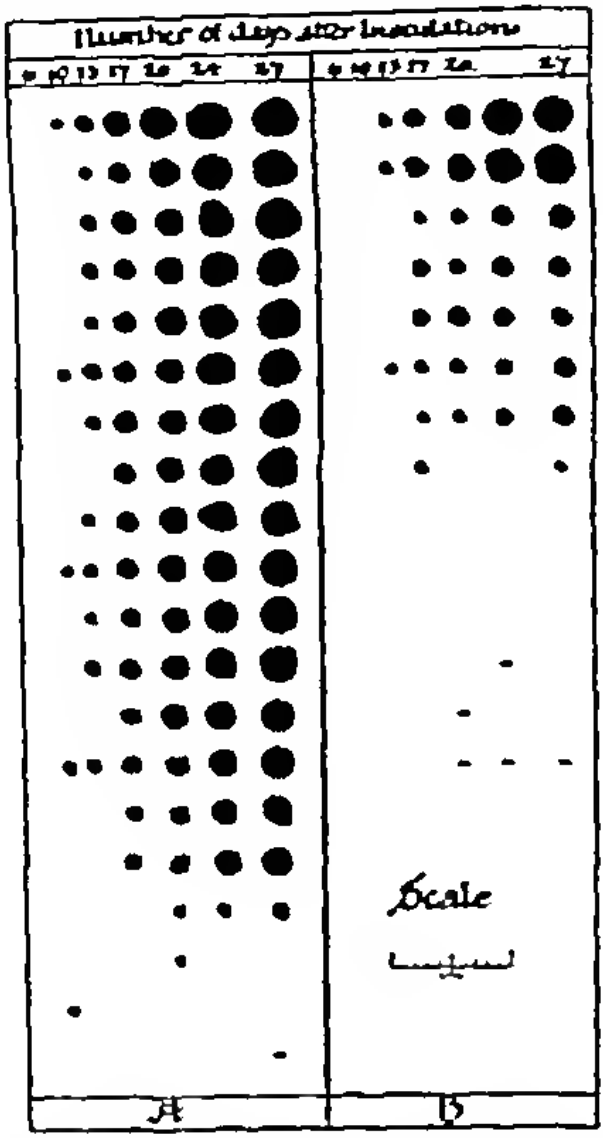
In view of the fact that immunity seems to be caused by degenerating but not dead tumour cells the products of autolysis of these cells clearly invited attention and a great deal of work has been done upon the subject. Chambers and Scott (1924) state that On the whole the results have been very uncertain and contradictory and no good evidence has yet been produced showing that autolytic extracts can cause immunity' Their own work was undertaken in order to determine whether extracts capable of influencing tumour growth could be prepared from tumour cells themselves by radiation, autolysis under varying conditions and for different times and fractional precipitation by means of acetone

Having regard to the work of Carrel (1913) and Drew (1922–3) upon the stimulating effects of certain tissue extracts in promoting the growth of cells *in vitro* the possibility of the coexistence of stimulating and retarding factors was borne in mind Bashford Murray and Haaland (1908) showed that by using emulsion of mammary tissue increased sensibility to tumour growth could be caused in mice

Chambers (1924) carried out a series of experiments on rats in which small engrafted tumours were completely excised either 24 or 72 hours after exposure to a lethal dose of radiation in the living animal Test inoculations made subsequently showed that the degree of protection afforded increased with the time the irradiated tumour was allowed to remain in the body before excision When that time was only 24 hours the degree of immunity was only small.

Chambers and Scott (1924) published the results of a series of experiments based upon the hypothesis that the immunity producing antigen was produced in decadent tumour cells probably by the action of intracellular enzymes They concluded that not only was this body unstable and evanescent but that it was associated with another body of diametrically opposite properties causing stimulation of tumour growth. One of their most striking experiments may be described The tumour (J.R.S) was removed and given a lethal dose of X rays according to the standard evolved by Chambers,

Russ and Scott (1922) It was kept at room temperature overnight it was then minced and 10 c c of this tumour were shaken with 50 c c of Drew s fluid for 6½ hours at 42 C a few drops of toluene having been added as a preservative



A. Controls. B Experimental

FIG 31 Showing the effect of a dose of irradiated and autolysed tumour tissue before tumour growth. (Chambers and Scott, 19 4.)

The mixture after incubation was centrifugalized and 0·5 c c of the turbid fluid inoculated subcutaneously into both flanks of 20 normal rats Four weeks later the 16 surviving animals together with 20 controls all apparently in good health received a test inoculation. The results are set out in the accompanying diagram where each horizontal line shows

the growth of a tumour in a control and in an experimental rat The tumours in the experimental animals are much smaller than those in the controls the average final volume in

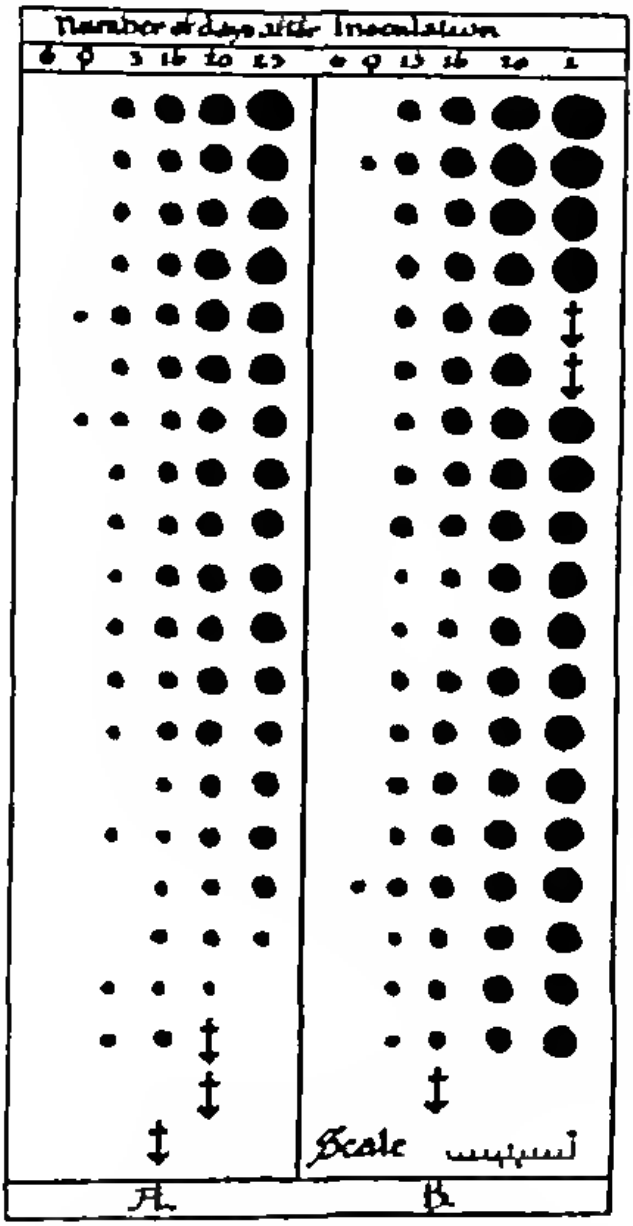


Fig 32. Stimulating factor

A. Controls. B Experimentals.

The two figures show the effect of injection with growth-stimulating and growth-inhibiting factors prior to tumour grafting, as mentioned in text (Chambers and Scott, 1924)

the experimental series being 4 38 c c as compared with 34 3 c c in the controls

The results are too uniform and too consistent to be the consequence of extraneous accidental conditions and one is led to the conclusion that a substance inhibiting tumour

growth was present in the injected autolysate of the irradiated tumour

Various modifications in technique were tried with the

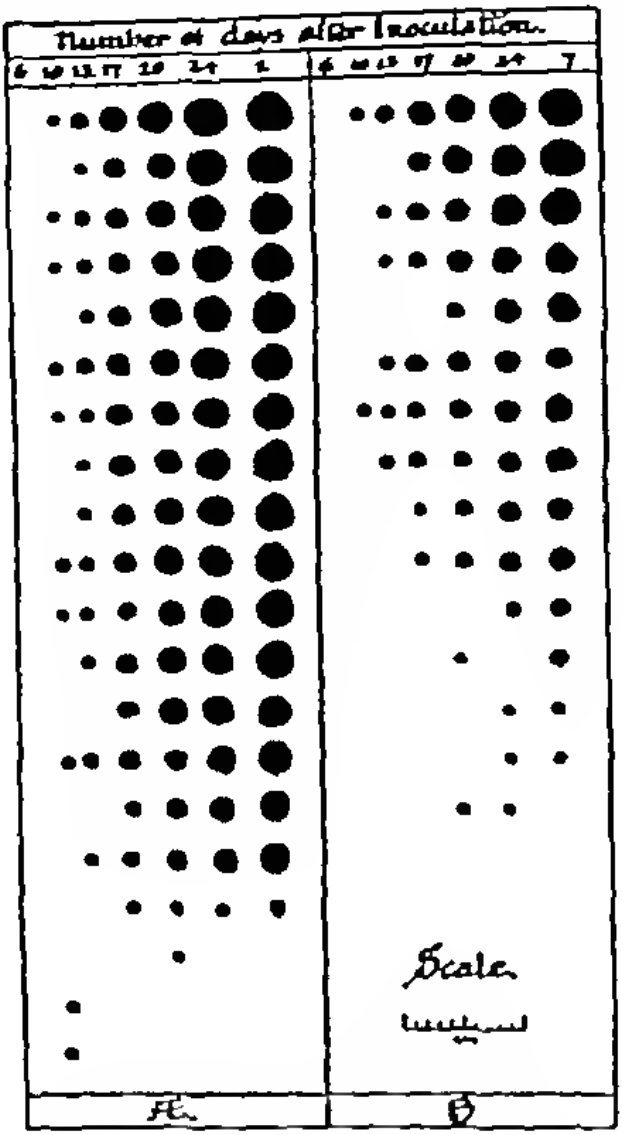


FIG 13. Inhibiting factor
A. Controls. B. Experimentals.

idea of obtaining improved results but the same remarkable uniformity was not again obtained. The periods of autolysis were varied as well as the time elapsing between injection of the autolysate and the test inoculation Chambers and Scott explain the discrepant results as follows

It appeared in all these experiments, when the whole of the fluid was inoculated, that we were introducing stimulating substance in

addition to antigen if the latter were present at all. With complete lack of knowledge of the effect of a mixture of these bodies, and of the rate of excretion of the stimulating substance we were not likely to obtain consistent results by continuing on these lines. Separation of the two bodies seemed, therefore essential to success.

They considered that the discrepant results might be due to the different intervals between the (intentionally) protective inoculations and the test injections and that in the case of the longer period the animals had succeeded in eliminating the stimulating factor Moreover with different periods of autolysis it seemed that with the 2-hour fluid a slight inhibiting effect was produced while with the 4-hour fluid a decided stimulating action was manifest

Experiments upon the autolysis of non irradiated tumours by precipitation with different strengths of acetone re-solution of the precipitate and subsequent test inoculation of the extract showed that such extracts might have either tumour stimulating or tumour inhibiting powers according to the method of preparation. It may however be said that the stimulating factor was more often manifest than was the immunizing factor The two accompanying diagrams show the effects of inoculation with extracts the one predominantly stimulating the other predominantly inhibiting

In a later communication (1926) the same writers state that the stimulating agent is a fairly stable chemical substance resisting exposure to a temperature of 100 C for 10 minutes and capable of being formed by the autolysis of tumour tissue in N/20 HCl and also in anaerobic cultures of J.R.S. grown under the conditions specified by Gye (1925) They regarded both the stimulating and the inhibiting factors as being derived from the nuclei of the tumour cells

Further investigations by Chambers and Scott (1932) upon the formation of tumour inhibiting antigens are summarized thus

(1) Experiments show that J R S deprived of its blood-supply and kept at blood temperature undergoes a transitory change, during which extracts of it have immunizing properties.

(2) The immunizing property develops for a short time with increasing potency and then disappears; it is apparently due to changes in the tumour cells set up by defective oxygenation

In view of the action of radiation in disturbing oxygen metabolism the second of these conclusions is of considerable interest The probable nuclear origin of anti-growth and growth-stimulating factors is also of obvious interest in this connexion It has already been shown that some of the earlier effects of radiation upon nuclei is to produce changes which histologically present appearances similar to those occurring in autolysis If the immunity producing antigen is as it seems to be unstable and evanescent it is clear that radiation may influence many factors which have possible connexion with its production In particular there are the questions of the permeability of the nuclear and cell membranes of hydrogen ion concentration and of variations in the oxygen metabolism of the cells The very different effects of radiation on different types of normal tissue have already been mentioned very possibly considerable variations may also exist in the degree to which different types of tumour are influenced. Little if anything is at present known of the action of radiation on the different stages of autolysis from natural proteins to amino-acids We are at a similar loss for accurate knowledge regarding its influence on the various stages of autolytic degeneration of the nuclear structure with which Chambers and Scott regard the tumour inhibiting and tumour-stimulating factors as closely associated

It may be noted that with the injection of irradiated material immunity was only produced when the inoculation was made under what may be termed ideal conditions By this is meant the careful distribution of the inoculum so that the fragments come into intimate relation with the essential normal tissues of the inoculated animal Implantation of a large irradiated mass has usually been found ineffective as has also the inoculation of well-divided material so injected as to form a localized compact mass It is also interesting that as good an immunizing effect seems to be produced by the inoculation of quite a small fragment as of larger quantities The essential feature of success appears to be that the implantation is made in precisely the right situation as regards the reacting tissue of the injected animal

A cytological analysis of the cell reaction to implanted

tumours in rats and mice was made by Da Fano (1910 1912) Studies were made of the connective-tissue changes in carcinomata undergoing spontaneous absorption and also of the reactions following the inoculation of embryo-skin, of blood, and of tumour cells as well as of tumour material previously killed by freezing and mechanical disintegration

The general conclusions which he reached and which were subsequently confirmed by Ludford and other workers are that

1 Lymphocytes appear in large numbers around the site of inoculation during the development of immunity
2 Killed tissue produces no immunity and no lymphocyte infiltration.
3 When small groups of lymphocytes occur in tumours which are growing well they are only found in areas of spontaneous healing

It was noticed by Maximow (1928) that in inflammatory conditions large numbers of lymphocytes and monocytes pass out of the blood vessels and become transformed into larger cells which he termed polyblasts and which finally are indistinguishable from macrophages

The whole question of the nomenclature of these cells is very complicated but it would seem from Maximow's work that many types which have received special names are merely stages in the evolution of macrophages from 'lymphocytes All these various types of cell have the power of segregating vital dyes and therefore are classed as belonging to the reticulo-endothelial system Moreover experimental evidence indicates that when these vital dyes have accumulated in the cells there is interference with their immunizing power

Ludford (1932) investigated the effects of vital staining upon tumour immunity produced by the injection of embryo-skin

A batch of mice was immunized by injecting embryo-skin in the right axilla. These were divided into two groups. The mice of one group were vitally stained a week after the inoculation of the embryo-skin. At the end of the second week, both groups, together with a

set of controls, were inoculated in the left axilla with the transplantable adenocarcinoma 63 of this laboratory [1]

As a result of this vital staining it was found that the immunity produced by the embryo-skin inoculation had broken down.

The materials used by Ludford in his *intra vitam* experiment were the dyes trypan blue and vital new red as well as colloidal preparations of aluminium hydroxide and of sulphur These are obviously of very different chemical nature the one feature they have in common in addition to their colloidal character is that they are segregated by certain cells of the animal body Moreover the doses in which they are effective approach the limits at which they are toxic Dyes which are not thus segregated—whether acid such as eosin and orange G or basic such as rhodamin B—have no effect in lowering immunity

The amount of lymphocytic reaction produced by tumour inoculation varies with the type of tumour introduced Thus taking the two tumours 206 and 63 of the I C.R.F Laboratory the results of reaction to implantation may be thus contrasted

Tumour 206.	*Tumour 63*
Ill-defined margin with extensive leucocyte infiltration.	Well-defined margin with edge clearly marked off from surrounding tissues.
Frequently regresses and causes concomitant immunity	Invariably grows progressively and does not produce concomitant immunity

Mice were inoculated with tumour 206 given five injections of trypan blue and tissue cultures were made It was found that the dye was segregated by the non malignant cells It was also noted by Ludford that in tumour bearing mice vitally stained with trypan blue the segregation of the dye by the non malignant cells surrounding the tumour was most marked in those tumours which were undergoing regression.

Some work by Foulds (1932) shows the importance of the reticulo-endothelial system in controlling the development

[1] I.e. the laboratories of the Imperial Cancer Research Fund.

of tumours Experimenting with the Brown Pearce rabbit sarcoma emulsions of tumour cells were injected into the ear vein. It was found that such injections led to the formation of metastases in much the same way as when the tumour cells were injected into the testis or leg and then allowed to develop In either case metastases were rare in the spleen and uncommon in the lung and liver Vital staining with trypan blue greatly increased the incidence of tumour in the spleen, lung and liver It would appear from these experiments that some organs resist the formation of metastases in much the same way as a local mechanism opposes the successful taking of tumour grafts Foulds is of opinion that in both instances the effect is due to the action of the reticulo-endothelial system The absence of metastases in, e g the spleen and lung is not due to absence of cancer cells. These were found microscopically in all the cases examined they only grew however when the reticulo-endothelial system was damaged In the other cases they were destroyed *in situ* before they had time to develop

A large number of experiments upon the blocking of the cells of the reticulo-endothelial system by the injection of materials which are segregated by them has been carried out In some cases this has appeared without effect on the growth of transplanted tumours while in others such growth was markedly increased. The explanation suggested is that when no effect was produced the dose of segregable material inoculated was too small.

An interesting experiment in which the effect of X rays upon tumour transplantation was investigated was that recorded by Chambers Scott and Russ (1920) A spontaneous tumour was found to have developed in the neck of one of the stock rats in their animal house It was found to be an encapsuled growth of the submaxillary gland [1] Autologous inoculations were carried out and transplantation attempted into 22 normal rats The tumour did not recur

[1] The authors described the tumour as closely resembling spheroidal celled carcinoma of the breast in the human subject so far as its histological features were concerned. Spontaneous carcinoma in the rat is rare and difficult to propagate by inoculation.

at the site of removal but large tumours developed as the result of the autologous grafting the inoculations into the 22 normal rats were unsuccessful. It was then decided to try whether such transplantation could be facilitated by exposure of the rats to X rays before the grafts were made

Seven rats were given a rather large dose of X rays—15 minutes exposure[1]—and on the following day the inoculations were made It was found that the tumour grew successfully in one animal subsequent inoculations in irradiated animals were more successful and comparisons with control non irradiated animals showed that tumour development and growth were much more marked in the irradiated rats

It had previously been shown by Mottram and Russ (1917) that when rats which were immune to sarcoma were given heavy doses of X radiation their immunity was temporarily broken down. The same workers, collaborating with Chambers and Scott (1919) showed that when rats are given *very small* daily doses of X rays they become less susceptible to subsequent inoculations of sarcoma

Zacherl (1929 1939) who has given a great deal of attention to the part played by the reticulo-endothelial system in affecting the growth of neoplasms considers that it is of the highest importance in the human subject as well as in transplantable animal tumours In a review of the subject made in 1930 he refers to the well known rarity of metastatic deposits in the spleen in cases of human malignant disease and also to the increase in tumour growth when tumour material is inoculated into splenectomized as compared with normal mice Also if the minced tumour material is mixed with ground up spleen or thymus tissue the tumour growth is retarded This was not found to hold when organs other than spleen or thymus were used

The difficulty of successfully implanting mouse tumours into rats is well known but if the rats are subjected to splenectomy and *soon afterwards* (1–2 weeks) inoculated with mouse tumour successful implantation is obtained If the test inoculation is delayed the resistance to the implanted

[1] This is about one half of the dose which the authors had found to be lethal for malignant cells.

mouse tumours is regained The situation is explained if we regard the reticulo-endothelium generally as developing a compensatory increased functional activity in consequence of the removal of the spleen. When the inoculation is made soon after splenectomy this has not had time to develop and so a successful graft can be made

Mention is also made in the same paper of the observation that if spleen and tumour cells are grown together in tissue cultures the growth of the tumour cells is impeded.

In the case of the splenectomized rats as already mentioned resistance to implanted mouse tumours is re-established after 3 or 4 months If then the animals are exposed to X rays and reinoculated, successful grafts may be made Exposure to X rays has again broken down the resistance of the reticulo-endothelial system

According to Brūda (1928) if rats were exposed to considerable doses of X rays implantations of mouse carcinoma were successful. If the doses of X rays were small the resulting tumours if successful at all did not grow so well.

According to Zacherl's personal clinical observation it not infrequently happened that rather active growth of carcinoma of the cervix followed intensive doses of X radiation given at one sitting smaller doses appeared to him on the other hand, to produce a more immediate effect in causing tumour regression. In the former case he considers the result as caused by too extensive damage to the reticulo-endothelial system

Throughout the experiments it appears that radiations have a *similar effect upon* the *reticulo-endothelial system* to that produced by the injection of substances which undergo segregation by its cellular elements and that interference with its functions results As to the question of direct stimulation of the reticulo-endothelial system by small doses of radiation, this must be regarded for the present as a question needing a great deal more research. Injury to one part may very probably lead to a compensatory hyper activity of other parts On the other hand if as suggested by Pfeiffer and Brūda (1929) a protective substance (mesenchymin) formed by the reticulo-endothelial cells is present in the blood and

lymph alterations in cell permeability due to radiation may accelerate its liberation and a consequent temporarily increased concentration in the body fluids The whole question is admittedly at present a highly speculative one but with increasing work upon the subject further light may be hoped for

The important question naturally arises as to whether clinical irradiation of tumours may produce a rise in the patient s resistance we certainly meet with cases which may suggest it These occur mostly in the advanced and inoperable cases Quite apart from the benefit to health caused by the cessation of discharges and haemorrhage the effect of radiation sometimes suggests that proliferation and metastatic growth is inhibited when the sites are remote from the site of immediate intensive radiation. The general lack of anything like consistent and definite inhibiting action is to be expected if as Chambers and Scott suggest the immunity producing body is not only unstable and evanescent but also intimately associated with a body of diametrically opposite properties Moreover the importance which they find attached to the necessity for ideal conditions in inoculations designed to produce immunity in animals would go far to explain its apparent non production in the human subject Macroscopic metastases are obvious enough but how often microscopic metastatic deposits are influenced by radiation of the primary neoplasm is another question. As mentioned, Foulds found them widely distributed in cases of the rabbit sarcoma with which he worked This distribution was seen in organs where macroscopic metastases do not commonly develop Damage to the reticulo-endothelial system was however followed by their development as macroscopic growths It was then not a case of non invasion of the immune organs but a local (?) protective mechanism which hindered their development into visible tumours It would not be expected from experimental evidence that well-developed metastases should undergo regression The ordinary immunizing processes have usually no effect on well-developed existing tumours

Of course improvement in general condition often follows

radiation even in cases which from any point of view are hopeless as regards cure Cessation of haemorrhage, of septic discharge and relief of pain, are all contributory factors no doubt There is also the possibility that improvement in the general health may increase the patient s power of resistance to the disease Against this must be set the well known clinical fact that neoplasms occurring in young well nourished subjects only too often run a course which is dramatic in its rapidity and fatal result In experimental animals implanted tumours do not grow so well in ill nourished or cachectic individuals

It seems that the whole question is intimately bound up with the problem of dosage and distribution of radiation, and the more these are made the subject of exhaustive study the further shall we advance in our knowledge of the cancer problem as a whole

CHAPTER VI

THE ACTION OF RADIATIONS ON MALIGNANT GROWTHS

Although from time to time specimens of irradiated neoplasms had often been examined histologically the first systematic investigations upon the cell changes following a single exposure were those of Lacassagne and Monod (1922) It will, therefore be desirable to consider these in some detail before discussing some of the later work upon the subject

The first study was made upon a sarcoma situated in the abdominal wall of a bitch close to the margin of one of the mammae The growth which was of the size of a fist was removed surgically as far as possible but as invasion of the subjacent tissues was extensive the residual growth was given a single dose of X rays after which portions were removed from time to time for histological examination The details of irradiation were given as

Standard Coolidge tube	
Distance from anticathode	8 cm
Aluminium filter	12 mm
Spark-gap	26 cm
Duration of exposure	5 hours 90 min.
Total dose (measured at skin port of entry)	20 5 H

The animal anaesthetized with morphine was placed so that the beam of rays traversed the remains of the growth from side to side thus avoiding as far as possible irradiation of the deeper organs Eleven specimens were removed for examination (biopsy) the first before irradiation and the subsequent ones immediately afterwards then after 22 and 42 hours and after 3 4 5 7 9 11 and 14 days

The histological findings were summarized briefly as follows

(1) *Before irradiation* The growth was a spindle-celled sarcoma; the fusiform cells were arranged in bundles, with central elongated nucleus, containing from one to four nucleoli, which exhibited strongly basophile characters; numerous mitoses were present

of which the greatest number were regular; some atypical forms were seen, which were most frequent in the vicinity of the ulcerated surface where there was also evidence of polymorphonuclear infiltration. The cells were embedded in a collagenous network, by which they were united into bundles, while at the same time the bundles were separated by wider meshes of the same collagenous fibres

(2) *Immediately following irradiation.* The general appearance of the specimen is unchanged, as compared with the non irradiated, but there is almost complete disappearance of mitotic figures, and in some places there is evidence of local cell necrosis with fragmentation of the chromatin, which is dispersed in the condensed cytoplasm

(3) *22 hours after irradiation* Mitotic figures have reappeared, and are as numerous as in the non irradiated specimen. Many however are abnormal, and none are seen in anaphase or telophase. Necrotic cells more numerous than in preceding specimen.

(4) *42 hours after irradiation* The superficial area of many cells is greatly increased, though the cytoplasm appears less dense. The collagen fibres appear much thickened Very few normal mitoses are seen there are numerous cells in prophase, the majority being in metaphase A few also in the terminal phases, but these are very rare and markedly atypical Many cells showing atypical mitoses also show degeneration

(5) *3 days after irradiation* The abnormal features seen in the previous specimen are accentuated The cells appear diminished when compared with the collagenous matrix many show cytolysis, and in some cases the meshes of collagen fibres are empty or contain only cell debris. Mitoses are abundant in proportion to the number of cells they are, however for the most part abnormal, though a few apparently normal forms are seen. Several multinucleated giant cells are seen The nuclei of these vary in number from 8 to 15 they are smaller than normal, of unequal sizes and irregular outlines.

(6) *4 days after irradiation.* The abnormal features continue to increase in prominence There is an increased disappearance of cells, while those remaining are often multinucleated and very large The number of empty spaces in the collagen network has increased, though some are filled with cell debris. Mitoses, although abundant, are abnormal.

(7) *5 days after irradiation* The leucocyte invasion appears general and extensive The meshes of the collagen network are mostly empty or containing debris, such cells as remain are abnormal and degenerate A few mitoses are seen in the surviving cells, but they are irregular and abortive In many places the meshes of the collagen reticulum are filled up with leucocytes. Such cells

as are present are lacking in definition and staining power Blood vessels are usually thrombosed.

(8) *7 days after irradiation.* Only a few tumour cells left and these highly abnormal.

(9 10 11) *9 11 and 14 days after irradiation* Progressive disappearance of even abnormal tumour cells, and progressive degeneration of collagenous reticulum

A recurring nodule which had grown up at the edge of the operation wound showed histological characters identical with those in the non irradiated specimen

The sequence of events after radiation is obvious enough Cessation of mitosis with cell degeneration followed by renewed but abnormal mitotic activity Gradual disintegration and disappearance of neoplastic cells in which also the collagenous reticulum participates

A series of cases of irradiated human tumours were also examined, and the details of five are recorded in the paper now under discussion.

Case I Carcinoma of cervix uteri Fungating tumour filling the vagina. Radon in cervical canal, in each fornix and four needles implanted in the growth (24–29 November) total dosage 53 24 M.c d. 20 00 in uterine canal 33 18 in vagina and implanted in growth.

Before irradiation the section showed thick strands of carcinoma cells showing the beginning of epidermal differentiation the basal type of cell is however the predominant one Some cells show giant or multiple nuclei.

(1) *4 hours after beginning of irradiation.* The only alteration from the normal is the infrequency of mitoses, which is very marked

( ) *48 hours* Mitoses again numerous, but in many cases abnormal.

(3) *3 days* Appearances much the same as in the preceding specimen.

(4) *4 days* Cells showing cytolysis are frequent in the strands of cancer cells. The width of these strands is greatly reduced, in spite of the increased size of the remaining individual cells.

(5) *6 days after beginning of treatment, and 48 hours after removal of radon* The strands of malignant cells are narrowed: the cells so diminished in number as to be reduced to a few layers. Individual cells show irregularities in shape and size; mitoses are frequent but abnormal and obviously degenerative

(6) *7 days after beginning of treatment and 3 days after cessation of radiation.* The lysis of the malignant cells is very marked. There are fragments of the original strands of cancer cells still to be

made out the majority show cytolysis the others are irregular in shape and size, and no more mitoses are seen.

Case II Carcinoma of cervix uteri Fungating tumour growing from the posterior lip of the cervix. Histologically extensive epithelial downgrowths under the tissues showing commencing differentiation of epithelioid type No keratinization Numerous mitoses mostly normal. Radon in cervical canal and in vagina one applicator being placed in each fornix and one mesially (30 March–3 April) total dose 59 52 MLo d. 25·56 in uterine canal 23 96 in vagina.

(1) *24 hours after beginning of irradiation* Mitotic figures greatly reduced in number and quite absent from the basal layer In the other layer a few abnormal mitoses are seen.

(2) *48 hours* Mitoses, though less numerous than before the beginning of treatment have again become frequent but nearly all are abnormal. In the centre of some of the strands of malignant cells the maturation of the cells is shown by the accumulation of cells showing hyaline changes, or changes more or less akin to keratinization.

(3) *3 days* Many abnormal mitoses, and many cells are seen with two or more nuclei with irregular outlines.

(4) *4 days* The day of removal of the radon applicators. There is marked narrowing of the strands of cancer cells: there are fewer mitoses, and those abnormal, but of the cells remaining, many are of monstrous forms and multinucleate Others show cytolysis, and in the centre of the strands changes of a keratinous nature are to be seen.

Two further cases of uterine carcinoma are described one of them showed a more advanced stage of epithelial development while in the second all stages of normal epithelial development up to marked keratinization were distinguishable There are no essential differences in the general nature of the changes produced by radiation from those previously described

The last case described by Lacassagne and Monod was that of a metastasis in the pre-auricular lymph gland, secondary to a squamous-celled carcinoma of the forehead, which had been locally cured by operation. The secondary growth had become greatly enlarged and ulcerated. Histologically the growth showed an advanced stage of epidermoid differentiation, with a marked predominance of keratinized elements.

There were numerous mitoses in the peripheral areas but these were frequently abnormal asymmetrical and multipolar The mass was treated by inserting twelve radium needles the dosage corresponding to 15 *micro*curies destroyed per needle per hour

(1) *3 days after insertion of needles* The structure is completely changed and the columns of malignant cells broken up The remaining cells are abnormal enlarged, with nuclei of irregular structure and outline and often accompanied by smaller atypical nuclei Mitoses are frequent but abnormal, and keratinization marked

(2) *6 days* The features of the preceding specimen are exaggerated In many places there is complete cell destruction and where viable cells are present mitotic figures are rare.

(3) *8 days* Neoplastic degeneration is still further advanced and most cells appear dead The survivors, greatly enlarged and abnormal, show extensive masses of keratinous material

Nine days after the beginning of treatment the radium was removed the total dose being 38 88 M.c.d Two days later a specimen showed complete degeneration of epitheliomatous elements keratinized debris was present around which were giant cells

The general effects of radiation upon these various human neoplasms are thus summed up by the authors

1 During the first 24 hours there is disappearance of mitotic figures and cessation of reproductive activity

2 Mitosis is gradually resumed and increases until towards the end of the second day All mitoses however are and continue to be, abnormal. In some cases, especially in the first few days, some apparently normal mitotic figures continue to appear the normal terminal phases are however absent suggesting that the cells died before the completion of division. In other cases the mitotic figures are quite abnormal, and at the conclusion of 3 or 4 days there show all kinds of mitotic monstrosities The degenerative process increases with lapse of time and the cells die off

3 In these carcinomata, as well as in the sarcoma first described degenerative mitotic processes appear to be the essential element in causing destruction of malignant cells As the process of abnormal mitotic division progresses they gradually diminish in numbers until they eventually disappear

4 This process can be seen in all the types of cancer examined in those growths where there is a normal tendency toward the complete evolution of the epidermal type this maturation of the cells seems to be actuated by irradiation

Lacassagne and Monod give a review of the results obtained by other workers who have examined malignant tissues after irradiation—Scholtz Apolant Martini, Clunet and Rubens-Duval—as well as of some of the effects obtained with non malignant tissues Putting all the work together they are of opinion as already said that the curative action of radiations in neoplasms is in the first instance determined by mitotic changes and degeneration

Cantu and Donaldson (1923) gave the results of the histological examination of specimens from irradiated carcinomata of the cervix uteri Their general conclusions are as follows

(*a*) That the introduction of 173 6 mgm of radium element into the cervical tissue for 24 hours is capable of causing a complete disappearance of the growth from the cervix within a few weeks whereas the same application for 8 hours produces little or no effect upon the quantity or appearance of the growth

(*b*) That definite series of changes in the carcinoma cells can be demonstrated after irradiation leading up to the destruction of the cells

(*c*) That little or no effect is produced in affected iliac glands if the cervix is irradiated.

(*d*) That as changes in the malignant cells take place before the formation of fibrous tissue the latter is not the causal agent in the disappearance of the growth.

(*e*) That carcinoma cells are more vulnerable than the uterine musculature but that nevertheless, in the latter local atrophy and fibrosis take place at a later date

Cytological studies of irradiated human cancers have been made by Ludford (1930) In general the type of nuclear change is similar to those described in animal tumours when dealing with the nucleus In describing an irradiated squamous-celled carcinoma of the lip he draws attention to the contrast between the great effect of the radiation upon the malignant cells and its small effect upon the normal epithelium Unfortunately he does not exactly specify the location of the radium needle as regards the normal and neoplastic tissues respectively The needle contained 2 mgm of radium

element and was left in position for eight days the tissue removed for histological examination was all taken in the area of 1 cm round the needle Neither the screenage nor the material of which the needle was made were recorded assuming it to be of the ordinary modern type it was presumably of platinum iridium and about 0·5 mm. in thickness The changes observed in different malignant cells were

1 Great enlargement of cells the nuclei showing irregularity of outline alterations in the chromatin, and accumulation of basi-chromatin in the nucleoli.
2. In some cells the chromatin is collected into droplets beneath the nuclear membrane
3 Lobulation of the nucleus accumulation of modified chromatic material into droplets These do not stain by the Feulgen method a few small scattered masses do however so stain as do also a few areas in the other cells.
4. The nucleus contains a large number of separate droplets of oxychromatin and basi-chromatin.

It is pointed out that this form of degeneration is unusual and has not been observed in tumour cells during autolysis

The summary of the conclusions reached by Ludford is thus given

1 The comparative freedom from the effects of radiation of the normal epithelium, although it was in an area of 1 cm. round a needle containing 2 mgm. of radium for eight days.

2 The radiations appear to have had a specific action upon the malignant cells All the cells were within an area of 1 cm from the source of radiation.

3 A large proportion of malignant cells have not been actually destroyed. Many of the undestroyed cells are so degenerated that they may not recover There are other malignant cells which may either recover or may never become capable of multiplication, having lost their malignant character

4 It cannot be said that radiation had increased the number of lymphocytes, polymorphs, or macrophages in the radiated area since precisely similar pictures can be seen in non irradiated squamous epitheliomata of the lip

5 The type of degeneration induced by radiation of the malignant cells is an unusual one

6 Abnormal mitoses has actually occurred during the radiation.

Mention has already been made of the fact that a certain minimum intensity of radiation is necessary to cause inhibition of mitosis in tissue cultures In Canti and Donaldson's work, clinical experience confirms what common sense would suggest that a sufficient dose of radiation must be given to ensure complete disappearance of the growth. The determination of the minimum dose necessary to produce this effect under various experimental conditions has necessitated a considerable amount of work upon the 'lethal dose This can be considered under two heads as regards animal experiments The lethal dose administered *in vivo* is the least amount of radiation which will produce the complete regression of a tumour left *in situ* while *in vitro* it is the least dose which when administered to a portion of excised tumour, will prevent its growth when injected into the flank of another rat

For the determination of the lethal dose *in vitro* Scott employs a slice of selected tumour tissue about 1 or 2 mm thick which is placed inside a thin mica cell sealed with sterile vaseline—the whole procedure is of course conducted under conditions of complete asepsis A large experience of the probable effects of the X ray beam used under standard conditions has made it possible generally to have some idea of the time of exposure which will be necessary As a rule four specimens are irradiated at once within the same area of radiation Half way through the exposure the slice of tumour is turned so that the former upper and lower surfaces are now reversed. Since the total area of the irradiated field is large enough very considerably to overlap the small area in which the capsules are placed, it follows that all the tissue elements must receive a uniform dose of radiation since the order of the duration of the exposure is usually about 180 minutes in all the specimens being placed at a distance of 30 cm. from the anticathode and the spark gap being 10 cm measured between spheres 5 cm in diameter

For the same type of tumour it is found

1 That the lethal dose given *in vivo* is slightly smaller than that given *in vitro* also when the *in vivo* dose includes the surrounding tissues (i.e the radiation is

not strictly limited to the tumour by lead screenage) the dose is much smaller than when the tumour alone is irradiated

2 With all the rat tumours experimented upon Scott finds the difference in lethal dose is not greater than 25–30 per cent The Rous chicken sarcoma is on the other hand, unaffected by 7 times the ordinary lethal dose for rat tumours whether the radiation be *in vivo* or *in vitro*

3 The amount of radiation necessary to constitute a lethal dose depends upon the wave-length. Although experience may suggest the order of exposure necessary each case must be investigated separately

4. In irradiating tumours for the estimation of the lethal dose it is found that the first 50 per cent of the necessary dose has but a comparatively slight effect the last 25 per cent of the time of exposure seems to be expended in overcoming the resistance of the more refractory elements to radiation Of course there is another possibility which is that some animals are much more susceptible to engrafted growths than are others Under these conditions a growth might require more radiation to render it incapable of taking than when implanted into less susceptible animals In the susceptible rat there may be more favourable soil for the implant to grow in The prolonged and uniform radiation to which a slice of tumour about 2 mm in thickness is subjected would certainly at least suggest that resistance on the part of the inoculated animal may be no unimportant factor in determining whether the graft takes or otherwise

Russ and Scott (1929) endeavoured to ascertain experimentally the minimum efficient intensities of radium and radon tubes for practical use A concentration of 1 mgm radium element per centimetre length of tube is one which is in common use the problem was to ascertain how far this could be reduced when the lessened concentration of radium was compensated by increased length of exposure Jensen's rat sarcoma was used for most of the experiments The

thickness of the wall of the platinum tubes was 0·3 mm. in most cases It was found that if the concentration falls below a certain amount the irradiation has very little effect even on cells quite near the tube and even although the tube is left in place for quite a considerable time

If a tumour has had a tube of radium or radon inserted for just the length of time which would cause it eventually to disappear but instead of removing the tube and awaiting the natural course of events small pieces are taken from the edges of the tumour and inoculated into young rats, it is found that a large percentage of these transplants will eventually grow although, had the tumour been left intact in the original animal it would have disappeared This applies to pieces taken from the edges at right angles to the centre of the tube which have received the maximum amount of *irradiation of any of the peripheral cells These transplants* are often very slow in beginning to show any signs of growth, but once they have begun they grow steadily although as a rule at a much slower rate than the normal.

There are of course many possible explanations for this. It is not known what part the surrounding tissue of the host plays in the disappearance of a tumour and of course the vascular conditions in the case of the transplants into young rats are entirely different

The general impression gained from these experiments was that the effect was a fairly direct one growth being prevented if the dose is large enough but if the intensity of radiation falls below a certain limit the tumour cells are able to some extent to cope with the situation Although the rate of growth may have been retarded for a time, the tumour will eventually grow

To ensure the disappearance of a tumour *measuring* 10 by 10 mm a short radon seed 5 mm in length in a platinum screen 7 mm long and 0·3 mm thick, containing about 2·0 millicuries of radon appears to be efficient This corresponds to a concentration of 4 0 millicuries per centimetre of active length In many cases tumours were found to have disappeared with smaller intensities

With increased active length of the tubes the intensity of

radiation can be reduced A concentration of about 0 6 mgm of radium element per centimetre of active length left in for 11 days can produce a lethal effect in a tumour measuring 14 by 12 mm The length of the tube was in this case 1 5 cm In this case the concentration of radiation was less than a quarter of that employed in the 5 mm radon seed when the experiment lasted 10 days The number of milligramme-element hours in this case was 138 and was definitely less than that (about 220) corresponding to the dose given by the 5 mm radon seed applied for 10 days

It was found that practically all the tumours treated with radium or radon grew for at least a few days after insertion of the tubes the rapidity with which they disappeared varied enormously

The experimental fact that tumour cells irradiated while growing in a rat and left undisturbed after radiation are much less likely to grow than if they are removed and injected into another rat certainly suggests that the environment of the implant is not without effect Russ and Scott (1927) investigated the effects of previously irradiating the area into which an implant is made Rats were completely screened with lead 2 mm thick which was sufficient to stop the X rays used in these experiments Two apertures were cut in the lead through which the X rays could reach the skin of the animals The rats were given measured doses of X rays while under an anaesthetic In some cases they were inoculated with Jensen's rat sarcoma before exposure to X radiation more generally however this was done three or four days afterwards The site chosen for the inoculation was as nearly as possible midway between the two irradiated sectors A large number of experiments all went to show that growth was more marked in the non irradiated than in the irradiated areas In many cases when the irradiated area was eventually invaded by the tumour it was found that the growth was much flatter than in non irradiated regions The experiments as shown in the various charts drawn up by the authors are remarkably striking

In all the experiments the X rays were generated from a Coolidge tube with an alternative spark-gap of 5 cm between

spheres 5 cm in diameter Under the same conditions of output and distance from 50 to 55 minutes exposure was found necessary to administer a lethal dose to Jensen s rat sarcoma The times of exposure given to the rats in the present series of experiments were 20 30, and 45 minutes in different cases The effects of these doses on the skin varied from partial epilation to slight blistering Experiments upon the same lines carried out with a rapidly growing haemorrhagic carcinoma and using an exposure of 30 minutes under the conditions employed before confirmed the previous experimental findings

It seems established from these results that as might most probably have been expected the condition of the site into which the implantation is made is a matter of very considerable importance Perhaps in this connexion it may not be inopportune to stress once more the importance of the method of implantation, and the necessity for an enormous amount of practice before definite experimental results can be claimed It is obvious that inoculated tumour material when broken up and well separated bears a much closer resemblance to the implant causing a macroscopic metastatic deposit than is the case when a bit of tumour tissue is taken more or less haphazard and inoculated without any special attempt at securing a proper distribution of its constituent elements

Some experimental results obtained by Russ and Scott (1929) on the subject of differential action next call for consideration From the earliest recorded instances of damage inflicted upon the skin by X radiation it has been appreciated that the more penetrating radiations had usually a less destructive effect Russ had already shown (1923) that exposure of the skin of a rat to a beam of X rays with a limited range of wave-length (0·45–0·30 Å U) produced certain reactions provided of course that the doses were large enough When the wave-length was diminished to 0·108 Å.U six times the dose required with the longer wave-length were necessary to produce the same effect With Jensen s rat sarcoma the same type of difference was noticed, only here it was found that in order to produce the same effect the dose

of the shorter wave-length had only to be increased to 2 8 times that of the longer These experiments are of importance as showing not only that different ranges of wave-length require different doses to produce the same effect in a given tissue but that the same ratio does not hold good for all tissues

In their later (1929) series of experiments Russ and Scott made use of X rays with a wave-length of about 0 10 Å.U and of the γ-rays of radium Both skin and tumour were exposed simultaneously so that both received the same dose A rat[1] with a tumour on each flank was covered with lead, a small perforation over one tumour and another high up on the back allowing the radiations to reach these two areas After exposure both tumours were excised and small fragments of the irradiated tumour taken not more than 2 or 3 mm. from the surface of the skin were transplanted into young rats Each of these rats also received an inoculation with a fragment of the non irradiated tumour of the same experimental rat Comparisons were made from the effects of radiation upon the exposed skin and upon the failure of growth of transplants from the irradiated tumour The dosage of X rays was estimated by the ionization[2] method. The results obtained were found to corroborate those recorded in the previous paper (1923)

Two definite conclusions were drawn from their experiments

1 That the reaction caused in the skin and tumour depends not only on the amount of energy absorbed by the tissues but upon the particular wave-lengths of the radiation.
2 The extent of this differential action appears to depend upon the nature of the tissue

Further it was noted that

If the Jenson s rat sarcoma experiments had been limited to the group of wave-lengths 0 45 to 0 30 Å U (soft rays), there would be nothing to suggest a selective action of the rays which could be taken advantage of therapeutically since about 1 7 times the permanent

[1] Of course a large number of rats were used in these experiments.
[2] The size and construction of the ionization chamber are of the utmost importance. Lack of attention to this may lead to totally fallacious conclusions.

depilation dose is needed to cause a lethal action on the tumour cells. When the wave-length is shortened to about 0 1 Å U., however only 0·75 of the permanent depilation dose is needed to kill the tumour cells, so that, not only for the short wave-lengths penetrating power but also because of the change in the right direction of the differential factor it seems it must be more satisfactory to use short wave-length radiations for any subcutaneous growths.

I cannot do better than quote the final passages of this very valuable and informative paper, since it is impossible to abbreviate them with any approach to a clear explanation of their conclusions

At the present time, it is not likely that any single explanatory theory of the biological action of X and gamma rays can be seriously propounded on account of the extraordinary diversity of these actions. It is, perhaps in the phenomenon of selective action that this diversity is best seen No single theory can be expected to fit all the facts that are known of the biological effects of these rays, but it is tempting to propose in a very tentative fashion an explanation of our experimental facts in terms of ionization gradients"

The outstanding physical effect of these rays is the ionization they produce Ionization doubtless occurs throughout the irradiated tissues electrons are liberated and their velocity depends on the wave-length of the exciting radiation. These, after all are the outstanding facts known on the physical side, and it remains to be seen how this difference in the velocity of the electron can account for the difference in biological effects when the amount of energy liberated is the same

In the case of tissue exposed to very short wave length radiation, the velocity of the electron is high and, when liberated from the cell, it passes through a considerable thickness of tissue before its velocity is quenched With longer wave-lengths the velocity of the electron is lower and the distance traversed much less. It is possible to examine this difference in greater detail; there is a lot of data at hand giving the varying degrees of penetration of electrons in terms of velocity

Lenard in his publication (*Heidelberger Akademie der Wissenschaften* 1913) says that the "ionization intensity" of electrons having a speed of 0·90 V is reduced 10 per cent in going through 0 03 mm. of aluminium If the speed is 0·6 V., as little as 0 001 mm. will cause this 10 per cent. reduction (V is the velocity of light).

By a careful series of absorption measurements involving more than 600 observations, we have got comparative data of tumour tissue and aluminium

In the accompanying table, the data have been collected which bear on those regions of the spectrum which have been used in these experiments In the first column, the speed of the electron is given,

corresponding to the greater part of the particular radiation used in the second column, the thickness of tumour tissue needed to cause a drop of 10 per cent in the ionization intensity of these electrons. The third column gives the thickness in the approximate number of cell diameters (tumour cells)

Average speed of electrons	*Thickness of tumour tissue needed to reduce ionization by 10 per cent.*	*Number of cell diameters*
γ-rays 0·90 V	0·328 mm.	14·0
Hard rays 0·75 V	0·006 mm.	3·8
Soft rays 0·60 V	0·006 mm.	0·4

It will be seen from this table that what may be called the "ionization gradients" must be very much steeper with soft rays than with harder ones. Although the cells of the body are always accommodating themselves to changes in their surroundings, it is not difficult to imagine that this must become very much more difficult if the changes are sudden, and a steep gradient of ionization may be equivalent to a sudden change. Though just as much actual energy is liberated in the tissues by short wave-length radiation, the ionization gradients are not so steep, and it may be that considerably more energy has to be expended in these cases to cause disturbances from which the cell cannot recover than is needed when using the rays of longer wave-lengths.

It seems possible too that the power of the tissues to tolerate indefinitely very weak radiation, which is below a certain intensity may be explained in the same way.

Strangeways and Fell observed that if chick embryos are irradiated before blood vessels have yet appeared they are able to tolerate very much larger doses of radiation than after the development of a vascular system. Once this has become established irradiation sets up characteristic changes in the vessels which become thrombosed and the death of the embryo results. It is therefore to be expected on experimental grounds that tumours with a developed blood supply should present differences in their response to radiation when compared with tissue cultures and that valuable as the results of the study of irradiated tissue cultures unquestionably are the behaviour of a tumour irradiated *in vivo* cannot be assumed as a necessary corollary from the behaviour of tumour or other cells grown *in vitro*.

In considering the action of X and γ-rays upon a mass of tumour tissue such as is met with clinically the effect upon

the blood vessels is doubtless a very important one Vascular endothelium and according to some authorities the sub-endothelial tissue are highly sensitive to radiation The result of this action is to obliterate the lumen of the capillaries and other vessels within the range of action of an adequate dose of radiation In the case of arteries and veins collagenous tissue in the vessel walls accentuates the action, while if collagen forms a prominent feature of the tissue in which the vessels lie the swelling which it undergoes as the result of exposure to radiation will add to the effect of the endo-vascular changes by external compression Clearly the total effect of these changes will be to effect a marked diminution in the blood-supply to the irradiated area, thus leading to diminution of oxidation and of nutrition Lack of oxygen will not only cause a shift of the pH of the protoplasm of the irradiated cells, but, as has been already mentioned there is reason for thinking that excess of carbon dioxide is capable of affecting mitosis. Meantime the direct damaging effects of the radiations are already at work upon the cell nuclei so that more or less severe injury is bound to result if the dose of radiation be large enough. It has been shown how nuclear division is affected with the formation of abnormal mitoses and the dying out of certain lines of cells in one or even two generations If a cell is sufficiently damaged autolytic processes will assist in its removal and here the shift of the pH towards the acid side will accelerate the process In this connexion also the effect of nutrition upon autolysis may come into play as was seen in the case of autolysis in the livers of fed and fasting cats (p 45) where the process was much increased in the fasting state There is yet a further way in *which a deficient blood-supply may increase autolysis* It has been shown by Schryver and others that *in vitro* blood serum has a retarding effect due either to the presence of auto-enzymes or to partial neutralization of acid formed during the autolytic process Russ and Scott *(1927) in* studying the effects of implanted radon seeds upon tumour and liver cells of the rat found that cells lying around blood vessels seemed to be protected to some extent from the damaging effects of the radiation. Colwell and Gladstone

(1933) exposed the livers of rats to autolysis when full of blood and also when emptied of blood as far as possible In the former case the vessels connected with the organ were ligatured *in situ* in the anaesthetized animal before the liver was removed while in the latter the operation was carried out so as to ensure the loss of as much blood as possible

Both the livers were set to autolyse after which they were fixed stained and examined histologically It was found that autolytic changes were decidedly less marked in the liver which was allowed to autolyse when full of blood

Yet one additional factor may have its part in accelerating autolysis namely increase in the permeability of cell and nuclear membranes whereby removal of autolytic products will be facilitated thus leading in turn to still further accentuation of the autolytic process

Autolysis of course varies in degree according to the organ concerned and it seems very probable that the effects of radiation upon the autolysis of different types of cell may vary greatly Intestinal epithelium for example has been shown to be highly sensitive to radiation and also to undergo greatly increased autolysis as the result of previous exposure to radiation Perhaps the explanation of this may simply be that the epithelium owing to its highly radio-sensitive character is readily injured and that injury leads to autolysis Owing to their situation every facility is offered for the removal of autolytic products From their normal physiological functions it would naturally be expected—as is found —that these cells should be unusually rich in enzymes concerned in protein changes so that when severely injured their autolytic degradation proceeds with unusual rapidity

There is another aspect of the radio-sensitivity of the intestinal epithelium which has been suggested by Chantraine (1924) and others as exemplifying the phenomena of radio sensitivity considered as a whole namely that radio-sensitivity is a function of metabolic activity If we consider the intestinal epithelium it is plain that it is always working somewhere about its maximum capacity thus contrasts with the condition found in the heart or lung where both organs normally work at very much less than the maximum of which

they are capable Metabolic activity may be shown either in such functions as digestion respiration muscular move-

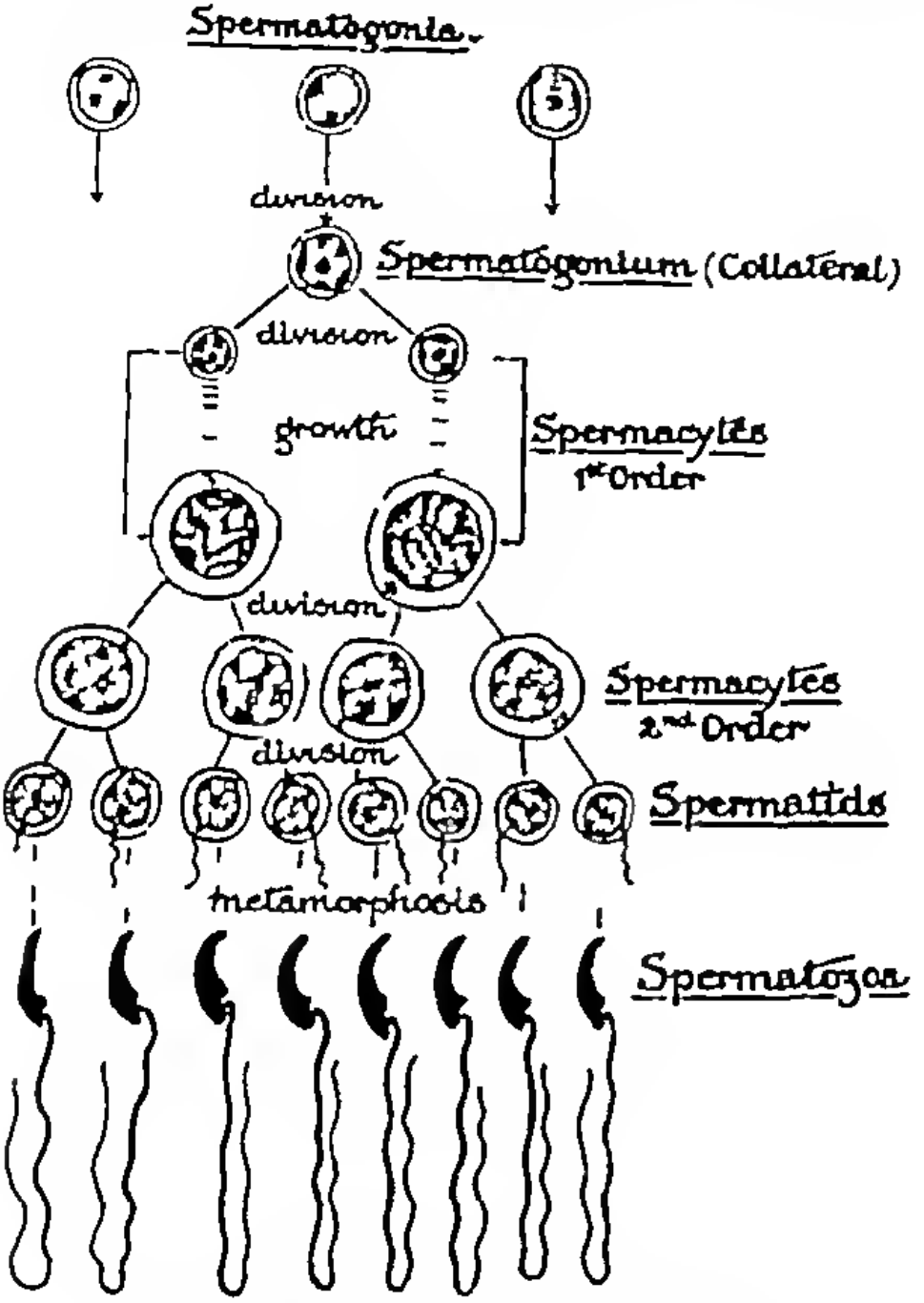


FIG 34 Development of spermatozoa from spermatogonia.
(Lacassagne, *Archives de l'Institut du Radium.*)

ment and the like or in nuclear mitotic activity such as occurs in the spermatogonia Here there is a diminution in radiosensitivity in passing through the various stages from spermatogonia to spermatozoa Post-radiation changes in the earlier stages are easily recognizable but mature spermatozoa them

selves can be irradiated without loss of mobility or obvious histological changes That they have undergone very marked alterations is shown either by non fertility or by the production of abnormal embryos when they are used to fertilize normal ova The fact that their histological appearance is unaffected is an important one since it proves conclusively that severe functional damage may co-exist with normal histological appearances Very probably with the increasing application of micro-chemical and micro-physical technique it will be found that changes demonstrable by such methods have occurred with ordinary reagents and methods of preparation no deviation from the normal can be made out

It has long been known that some neoplasms are much more sensitive to radiation than are others and various attempts have been made to correlate histological structure with radiosensitivity Differences in the response of tissues to radiation are markedly shown in two recent experiments on rats by Colwell and Gladstone (1933) In one of them a radium plaque containing 24 mgm of radium element and screened with 0 1 mm of silver was applied to the surface of the abdominal wall for thirty minutes the hair having previously been clipped Twenty four hours later there was no macroscopic evidence of change in the skin but on opening the abdomen the underlying intestine was found oedematous and showing obvious signs of inflammation. Marked changes of an inflammatory character were found on histological examination.

In another case the γ-rays from 123 mgm radium element (0·5 mm Pt filtration) were allowed to act for two hours Twenty four hours later the skin showed no visible changes but the intestine exhibited marked oedema and inflammation. It may be mentioned that in both these cases the cells of Auerbach s plexus were markedly affected by the radiation.

In general it may be said that the least differentiated types of cell are the most sensitive Thus Cutler (1922–3) recognized three main types

1 Those in which the cells are adult in character with a tendency to keratinization and cell nest formation. These are resistant to radiation

2 Those in which there is a moderate degree of anaplasia —the plexiform type—showing but few characteristic squamous features and a moderate degree of infiltration. This type is less radio-resistant than the preceding

3 Those showing a high degree of anaplasia with a complete absence of typical squamous characters and forming diffuse infiltrating growths. These are highly radio-sensitive

While the degrees of sensitivity to radiation are broadly in the above order those of malignancy are of course in the inverse order and although local regression may occur under the influence of radiation the early formation of widespread metastases may yet militate against a successful result. It is in such conditions as these that the question of the possible production of some form of immunity becomes an intensely practical one As said before when discussing the Brown Pearce rabbit sarcoma (p 128) although visible metastases do not commonly occur in certain organs e g the liver and spleen, this freedom from secondary growth is not due to non invasion of those organs by cancer cells Microscopic metastases can be demonstrated histologically and by damaging the reticulo-endothelial system by *intra vitam* staining the growth of well-developed secondary deposits can be brought about in situations which are normally free from them

The experimental work of Russ Chambers and Scott strongly points to the production of some factor or factors which inhibit tumour growth and that such factor or factors appear during the destruction of cancer cells If this is the case the treatment of neoplasms of highly malignant and metastasing type by small individual foci of highly filtered γ-radiation for a considerable period would seem to offer the best means for the formation of such antibodies in addition to this may be considered the continued action of the radiation upon cell and nuclear membranes thereby facilitating the passage of such substances into the circulation Obviously if such bodies exist their slow formation and escape from the injured cells will be more effective in favouring the destruction of microscopic metastases than will single highly

intensive doses whereby the cancer cells are rapidly destroyed The field is a very tempting one for speculation and perhaps in the fairly near future more light may be shed upon it by experiment and the statistical records of the results of radiation treatment

It is to Dominici (1907) that we owe the therapeutic use of highly screened γ-radiation as has been said the effect of β-radiation or of soft X or γ-rays is simply to produce localized necrosis Necrosis can also of course be caused by well-screened γ-radiation (1 mm. platinum + 2 or 3 mm rubber) if the dose is large enough and the time sufficiently prolonged. The aim of all modern radiotherapy is however to eliminate this indiscriminate caustic action and by adequate filtration and graduated exposure to administer such doses as shall have the maximum destructive effect upon neoplastic cells with the minimum of damage to normal tissues It is established that some cells and tissues are more radio-sensitive than others and all evidence points to the fact that cells of the same type show varying degrees of sensitivity at different stages of their mitosis and functional activity By suitably distributing the radiation both in space and time of application the chances of catching a cell in a highly vulnerable phase are much increased The steady exposure to small units of γ-radiation so disposed as to give as uniform a field as possible has been found in clinical practice to give the best results and the repetition of X ray doses is now generally preferred to the administration of a single massive dose With too intensive local dosage it appears probable that the reticulo-endothelial system may be so disorganized locally that as Zacheri records the first effect is a temporary overgrowth of the irradiated neoplasm By careful adjustment of dosage as regards time intensity and wave-length it is hoped to leave the best possible chance for the reticulo-endothelial cells to take part in the processes of defence and healing while the cancer cells are destroyed.

A great deal of work has been done on the metabolism of cancer cells notably by Warburg and others in the hope of establishing some specific difference in this respect between neoplastic and normal cells If such absolute differences

could be established it might very much facilitate the problem of treatment

Recent work by Crabtree and Cramer (1932-3) indicated that susceptibility to radium is not a fixed property of any given type of cancer cell but varies with changes in the environment and further that a primary effect of radium radiation on cells *in vitro* is a selective diminution in their respiration while glycolysis remains constant The broad conclusion which these authors reach in their last communication is that it is possible to produce experimentally great variations in the susceptibility to radium of cancer cells by acting on the respiratory mechanism of the cell Everything seems to point to alterations in the respiratory function as being one of the chief methods by which radiations act in addition to lack of oxygen from deficient blood-supply owing to vascular damage from the radiations, we have the direct chemical action of the radiations themselves, the reduction in glutathione the inhibiting or destructive action upon certain oxidases the action upon mitochondria (assuming these to be concerned in the processes of oxidation, as most people consider) and lastly interference with the permeability of the nuclear and cell membranes with probable interference with electrolyte equilibrium and so with the buffer reactions essential to respiratory processes

From a consideration of the preceding data it seems clear that the action of radiations upon both normal and neoplastic tissue is a complicated one In one type of tissue it is possible that one or more of these actions may be the dominant factor while in different types other methods of action may be the chief factors The fact that the relative radiosensitivity of skin and tumour tissue has been found by Russ and Scott to be reversed with different ranges of wavelengths seems a most important and valuable practical contribution to the subject By a judicious combination of X ray and radium treatment there seems a definite possibility of arranging dosage so as to obtain the maximum clinical benefit and the field for research in this direction seems most promising

In order to ensure the best clinical results from the

enormous amount of experimental work which has now been carried out extensive collaboration and co-operation between all classes of workers is essential

It is becoming increasingly clear that a department of Experimental Radiology with laboratories for physical chemical and biological research is an integral and essential part of any institution where radiotherapy is carried out Even a casual reading of the work which has been described in the present essay shows not only how immense are the problems which need investigating but how many are the different branches of science which must be called upon to provide their elucidation

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